1972

Influence of age on turkey muscle lipid deposition

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Influence of age on turkey muscle lipid deposition

by

Roger Maurice Wangen

A Dissertation Submitted to the
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INTRODUCTION

The growth and development of animal tissues is important to the food industry. Although the initial process of growth involves mainly bony tissue development, the food technologist is interested in the secondary growth and development stages which include protein production and lipid accumulation. It is generally accepted that protein production is abundant in younger animals, but in older animals its production begins to plateau. At this time, lipid accumulation increases at a rapid rate to provide the body with stored biological energy and insulation. The movement and presence of these components are important in relation to yield, composition and potential for deteriorative changes.

The poultry industry is interested in producing superior quality products in a profitable manner. Yield and composition of turkey carcasses, in relation to a food product, are of considerable interest to all people. Consumers judge the value of products through organoleptic means and approximate cost of edible tissue purchased. These factors are often influenced by the consumer who uses various preparation techniques, while certain other factors are definitely related to specified production techniques. Two influencing concepts include the age and the finish of the bird. Maturing birds deposit lipid, thus altering the organoleptic values of those tissues and the yield of total edible tissue.

In addition to carcass acceptability by consumers, further processed product quality is influenced by carcass composition. Cooking

muscle tissue often alters the amounts and types of lipid materials. Phospholipids, often involved in deteriorative processes, have been reported to increase in concentration in pork tissues as cooking time increases (Turkki and Campbell, 1967). The grinding of muscles exposes components to materials promoting the deterioration of the palatable and nutritious turkey products. Although lipids account for a small percentage of the product, their contribution can be very beneficial and/or of questionable value to the final product.

The purpose of lipid in muscle is manifold. Certain lipids contribute to the structural integrity of muscle cell membranes. Other lipids are deposited in muscle tissues to provide an energy reserve and an insulating tissue. All muscle lipids are involved in the organoleptic value of a meat product, and in certain meat animals, they contribute directly to the grade or quality measure of carcasses.

The presence of varying lipid types and concentrations affect meat flavor, texture, color, nutritive value, and deteriorative potential. The amount and type of lipid present in turkey has been related to many of these organoleptic values, but their relationship to deteriorative potential has not been completely reviewed. Knowledge of factors relating to the physiological deposition of lipid in various turkey muscles is essentially unknown. Since lipid deposition significantly influences the acceptability of turkey meat products through variable turkey muscle organoleptic properties, it becomes important to study these factors. Moreover, a characteristic that consumers recognize to predict eating quality of muscle tissue has not been extensively reviewed.

This investigation determined some information of turkey muscle lipids as the turkey advanced toward market age. As the bird matures, its body lipids change. Similarly, differences between muscles are apparent. Finally, this investigation attempted to elucidate the reasons for the variation in lipid content and class among muscles.

LITERATURE REVIEW

The physiological phenomenon of turkey muscle lipid deposition, utilization, and movement is dynamic and relatively unknown, although the contribution of lipids to food product flavor, texture and nutrition is well accepted. Variations in the lipid content of muscle occur between and within muscles. The more physiologically active turkey tissues are reported to contain greater concentrations of certain kinds of lipids (Marion et al., 1970). The relatively inactive Pectoralis major of the domestic Leghorn hen maintains a consistent level of lipid throughout the laying cycle (Wangen and Skala, 1968). A maturing domestic bird contains variable lipid factors which alter the composition of the final saleable product.

Transportation of Lipids

Lipids collect in tissues as a result of transport through the circulatory system. The principal form of transport between the adipose tissue and the recipient tissue is by albumin-bound nonesterified fatty acids while additional lipid transport forms include the lipoproteins, chylomicrons and erythrocytes (Evans, 1964). Hunter et al. (1965) suggested that lipid oxidized by tissues accumulated first in the adipose tissue and is released as non-esterified fatty acids for energy oxidation. Transfer of non-esterified fatty acids from the blood to muscle tissues is accomplished by carnitine (Fritz and McEwen, 1959). The absorptive ability of a tissue provides in part for lipid accumulation, and the blood flow to muscle tissues enhances the chances

for lipid accumulation. The blood supply to red muscle fibers has been reported to be greater than that to white fibers (Romanul, 1965; Reis et al., 1967). A threefold greater blood flow is reported to be found in Soleus muscles in contrast to the Gastrocnemius and is believed to be matched to its different function and metabolism (Beatty and Bocek, 1970). The Pigeon Pectoralis is reported to possess a copius blood supply (George and Naik, 1960), but they noted that capillaries to the broad fibers were fewer in number and that less blood reaches these fibers per unit area than in red fibers.

The movement of lipid to muscle tissues is regulated by certain physiological controlling areas (Havel, 1970). The plasma level and blood flow regulate muscle uptake of free fatty acids from the blood; approximately one-half is removed with each circulation. Lipase, the enzyme which hydrolyses lipids into products readily diffusable into blood, is regulated by the amount of cyclic 3'5' - AMP which is regulated by adenyl cyclase system activity. Catecholamines, norepinephrine, epinephrine and growth hormones are hormones regulating the activity of the adenyl cyclase system. Insulin acts as an inhibitor of hormone-sensitive lipase, but it increases the activity of lipoprotein lipase in the fed state, which reduces fat mobilization and increases uptake of triglycerides in the blood. The effect of fasting is different since the level of insulin secretion is decreased. A mitochondrial membrane transferase regulates fatty acid oxidation since it moves the acylcarnitine into the mitochondria from the cytosol. Electrical stimulation increased the free fatty acid uptake of rat diaphragm in vitro (Fritz, 1960) suggesting neural influences.

The uptake of lipids by tissues and their use for part of the tissue's energy requirement in different species has been reviewed by Issekutz (1970). The energy required by muscles to do work is generated from a chain of chemical reactions which is supported by a continuous fuel supply. The fuel supply includes carbohydrates and lipids primarily, and he notes that the expected contribution of each is difficult to assess. Both lipid and glucose metabolism occur in muscle tissues, and their metabolism are interrelated (Teppermen, 1962; and Gordon et al., 1963). These authors suggest that in the adipose cell glucose and lipid metabolism are interrelated in the following ways: (1) glucose provides acetyl CoA for synthesis of long chain fatty acids, (2) glucose provides acetyl alpha-glycerophosphate for fatty acid esterification and (3) glucose metabolism provides the cofactor, TPNH, necessary for certain reducing steps in fatty acid synthesis. Although both metabolic sequences occur in intact muscle, the metabolic sequences appear in different muscle fiber types.

Lipids in Fiber Types

Szent-Gyorgyi (1953) stated that red muscle fibers are capable of prolonged periods of contraction using oxidative metabolic reactions for energy production, and white muscle fibers use glycolysis to provide energy for their rapid and short contractions. The ratio of red and white fibers, therefore predicts the characteristics of the intact muscle.

Beatty and Bocek (1970), reviewing certain characteristics of red and white fibers, stated that Dubowitz and Pearse (1960) found smaller

diameters, higher mitochondrial content and greater oxidation activities in red muscle fibers than in white fibers. In addition, Beatty and Bocek (1970) mentioned the higher myoglobin content, greater lipase activity and larger triglyceride concentrations found in red muscles. The glucose-fatty acid cycle interrelationship is suggested to occur in heart and diaphragm muscle, but its presence in red and white skeletal muscle is questioned by the same authors.

The quantity of lipid varies among muscles and types of muscle tissues (Marion et al., 1970; Froberg, 1967: and Masoro et al., 1964). George and Naik (1959) teased apart red and white muscle fibers of pigeon Pectoralis and reported variation in lipid and glycogen content with depth of sampling. Surface muscle fibers contained larger proportions of glycogen and white muscle fibers, whereas red muscle fibers and fat content increased as sampling depth increased. In the domestic chicken, the breast muscles appear pale and/or white from gross examination and this muscle responds to many enzymes of the glycolytic pathway. In addition, the breast maintains a lower concentration of lipid when contrasted to thigh tissues (Marion, 1970). This leads to a question involving the characteristics of a muscle fiber and its ability to utilize, accumulate, and mobilize lipid. Variability in muscle lipid content may be the result of the animal's ability to oxidize lipid as a source of energy and/or the activity of mechanisms needed to accumulate lipid. Variations in glucose and fatty acid metabolism have been reported in lean and obese strains of mice (Hanson, 1964). Additional variations in lipid accumulation occur in the pector-

alis muscles of normal and dystrophic chickens (Butler et al., 1969).

The capacity of muscles to utilize lipid as a substrate in respiration is not completely understood. As reviewed by Fritz (1961), the low concentration of mitochondria in skeletal muscle leads researchers to think fatty acid oxidation is minimal, although quantitative data show fatty acid oxidation occurs in myocardial muscle. Opie (1969) and Masoro et al. (1965) reported free fatty acids, glucose and lactate are the major fuels for the heart, that glucose is most important after a meal, but that free fatty acids become more important in the post-absorptive state. Age of the rat appears to have an influence on the myocaridal capacity for fatty acid oxidation (Nikitin and Pashkova, 1963). They suggested advanced age reduces the activity of cardiac enzymes involved in lipid oxidation.

Pectoral muscles contain the most lipid of any tissue of the pigeon (George and Berger, 1966), and their lipid content is significantly decreased by electrical stimulation and forced flight. Vallyathan and George (1963) and Vallyathan and George (1964) reported that pectoralis fat content of migratory birds essentially doubled at the time of migration. Issekutz et al. (1965) reported dog muscle energy supplies differ in trained versus untrained dogs, and they concluded oxidative metabolism and adipose tissue reserve utilization occurred in the muscles of trained dogs. Untrained dogs were more dependent on anaerobic metabolism. Issekutz (1970) summarized the information on source of lipids as a fuel for muscle respiration, and concluded that both intramuscular and extramuscular lipids are used for energy production. However, the dominating mechanism is dependent on an unknown number of factors. Two fatty acid pools exist in tissue lipids (Volk et al., 1952 and Coniglio

et al., 1954), one of which is labeled more rapidly than the other. They suggest the pools are represented by constituent fatty acids, by fatty acids incorporated into membranes and by unbound soluble fatty acids.

The muscles of meat producing animals vary in their ability to utilize and accumulate lipid. Dark muscle of rainbow trout is reported to be more active in lipid oxidation than light muscle (Bilinski, 1963). Rat adductor muscle fibers use only one-fourth as much acetoacetic acid as diaphragm muscle fibers, but they use similar quantities of glucose (Beatty et al., 1959). Acetate oxidation by diaphragm muscle has been demonstrated to be 2.5 times greater than rat Latissimus dorsi (Fritz et al., 1958). George and Naik (1958) showed differences in the red and white fibers of pigeon breast muscle in lipid content and the effect of exercising. Grollman and Phillips (1954) suggested exercise increases the muscle's ability to derive energy from lipid sources by providing efficiency for lipid metabolism and obtaining energy for muscular work. Allen et al. (1967) reported differences in fiber proportions positive for enzymes capable of utilizing and accumulating lipids. Factors affecting these were sex, blood vessel size, and light and dark muscles. Accumulation of lipids in organs of experimental animals has been associated with degradation of enzyme systems in older animals (Weinbach, 1960; Leites, 1964). Bacterial aging is reported to be important to the degeneration of enzyme systems (Sorokin, 1964). Liver mitochondria from older rats showed a lower capacity for oxidation of B-hydroxybutyrate when compared with mito-

chondria from young rats (Weinbach, 1960). Older rats are reported (Leites, 1964) to contain lower quantities of lipase and nonspecific esterase in a number of organs. Due to greater concentrations of lipid found in organs of older rats and a lower level of lipolytic enzymes, he hypothesized a relationship about the "physiologic lipidosis" of the animal. The accumulation of lipid in tissues is a result of minimal oxidative processes in lipid breakdown instead of an increase in lipid synthesis by the cell (Takuzo et al., 1958).

Poultry Muscle Lipid Composition

Lipids commonly found in turkey muscle tissues provide functionality to the living tissue, and various degrees of quality in the food product. Neudoerffer and Lea (1968) reported the following composition of lipid from turkey muscles as a percent of total lipid:

	Breast Muscle	Leg Muscle
Total Lipid (% of wet weight	1.16	3.44
Cholesterol Ester	0.7	0.04
Triglyceride	27.8	57.0
Free Fatty Acid	2.3	3.4
Diglyceride	1.5	1.9
Cholesterol	6.0	3.0
Cardiolipin	2.0	1.0
Phosphatidylethanolamine	13.0	8.0
Phosphatidylserine	3.0	2.0
Phosphatidylinositol	5.0	3.0
Phosphatidylcholine	30.0	14.0
Sphingomyelin	4.0	2.0
Lysophosphatidylcholine	1.0	3.0

Fishwick (1968) reported breast muscle contained one percent lipid of

which 66 percent was phospholipid, and 2.4 percent lipid in leg tissue of which 37 percent was phospholipid. Of these phospholipids, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), plus phosphatidylinositel (PI), sphingomyelin (SPH) and lysophosphatidylcholine (LPC) accounted for 1.9%, 3.9%, 0.5% and traces respectively for breast tissue, and 3.2%, 0.2%, 4.9%, and 0.6% and traces prespectively for leg tissues. Marion (1970) reported 1.41%, 0.56%, 3.24%, 0.61% and 0.54% of PE, PI plus PS, PC, SPH and LPC in breast tissues, and 2.33%, 0.60%, 4.39%, 1.07% and 0.56% respectively for leg tissues. From these turkeys, total lipid accounted for 1.06% of breast tissue and 2.60% of leg tissue. Osborn et al. (1969) reported selected fatty acids found in lipid from turkey tissues, and all fatty acids were of carbon length 14, 14:1 (14 carbon chain with 1 double bond), 16, 16:1, 18, 18:1, 18:2, and 18:3. Dimick and MacNeil (1970) observed the presence of these fatty acids in turkey muscle lipid, but also noted the presence of fatty acids 16:2 and 20:4. Fishwick (1968) reported the presence of fatty acids with carbon length 14, 16 (an aldehyde), 16, 16:1, 17, 18 (an aldehyde) 18, 18:1, 18:2, 18:3, 20, 20:3, 20:4, 20:5, 24, 22:5 and 22:6. Fishwick (1968) observed the major fatty acids present in turkey lipid have carbon lengths of 16, 18, 18:1, 18:2, and 20:4, and that the long chain polyunsaturated fatty acids predominately occurred in the phospholipid fraction of the total lipid extract.

Specie Variation

Lipid commonly found in muscle tissue appears to vary with species. The concentration of lipid in turkey muscles is reported to range near

0.8 - 3.5 percent (Acosta et al., 1966; Neudoerffer and Lea, 1968). Depending on the specific muscle, Allen et al. (1967) reported lipid content of pork muscles varied from 3-10 percent. Hornstein et al. (1967) reported a 4-12 percent lipid content in different beef muscles. Phospholipid content also varies with specie according to Ansell and Hawthorne (1964). Greater concentrations of polyunsaturated fatty acids occur in poultry meat, particularly in turkey tissues (Chang and Watts, 1952). The characteristic fatty acids of species meat products carry variable taste sensations (Lushbough and Schweigert, 1960). Fishwick (1968) reports arachidonic acid accounts for 6-8 percent of the total lipid in fresh muscle. Fatty acids containing four, five, and six double bonds abundantly occur in lipids of turkey tissues, and occur primarily in the phospholipid fraction or as free fatty acids according to Fishwick (1968). In chicken tissues, Katz et al. (1966) reported greater quantities of polyunsaturated fatty acids in phospholipids found in muscle tissue than in phospholipids of skin and depot fat. In rat tissues, Widmer and Holman (1950) reported greater unsaturation in phospholipid fatty acids. Hornstein et al. (1967) reported the significant quantities of long chain polyunsaturated fatty acids in phospholipids extracted from beef muscles. Although most species maintain high levels of long chain polyunsaturated fatty acids in the phospholipid fraction, the total fatty acid content of lipid extracts varies between muscle tissue from pork (Allen et al., 1967), beef (Hornstein et al., 1967 and Waldman et al., 1968), broilers (Marion et al., 1967) and turkeys (Neudoerffer and Lea, 1968 and Fishwick, 1968).

Sex Influences

Some variability in the lipids of animal tissues has been reported to be influenced by sex of the animal. Female turkeys reportedly contain greater concentrations in muscle tissues (Davis and Brunson, 1963; Hartung and Froning, 1968), although male turkey muscle tissues exhibit greater susceptibility to lipid deterioration (Hartung, 1965; Osborn et al., 1969). Marion and Miller (1968) and Osborn et al. (1969) noted lipid variation in broilers due to sex. Twenty week old broilers possessed a lower oxidation potential than younger birds as evidenced by Marion (1969), although no association with sex was noted. In beef muscles, fatty acid differences were noted by Waldman et al. (1965) and Waldman et al. (1968). They observed greater amounts of saturated fatty acids in steer muscles, and larg-larger proportions of unsaturated fatty acids in heifer muscles.

Lipid Deposition

The turkey demonstrates variability in the accumulation of lipid at different sites in the carcass. Marion et al. (1970) reported large differences in total lipid observed in turkey liver, skin and depot fat. Considerable variation in lipid accumulation between two different muscles has been reported (Neudoerffer and Lea, 1968; Marion, 1970). Acosta et al. (1966) reported total lipid values for two muscle types of 16 week and one year old turkeys, and these values suggest variation in the total lipid content of turkey tissues. In laying hens, Wangen and Skala (1968) reported variable lipid deposition in thigh muscle tissues of maturing laying hens, but a lower consistent quantity of lipid was observed in the breast tissues. Marion and Woodroof (1965) reported greater concentrations of

lipid in thigh tissues of broilers. Lockhart (1960) stated that differences in muscle activity forms is dependent on connective tissue content, blood supply, myoglobin content and metabolic activity. Marion (1970) reported greater quantities of phospholipid in thigh compared with breast tissues. Marion et al. (1970) reported greater phospholipid concentration in the more physiological active turkey liver than in skin, breast, thigh or depot fat. Kaucher et al. (1943) observed variable phospholipid content among organs and tissues of differing physiological activity. In the chicken, leg muscle contains a greater concentration of phospholipid when compared with the less active breast (Peng and Dugan, 1965; and Marion and Miller, 1968). Neudoerffer and Lea (1968) reported similar findings in turkey muscle. Fatty acid distribution is reported to vary because of the type of tissue (Peng and Dugan, 1965; Katz et al., 1966; Mickelberry et al., 1966; Osborn et al., 1969; and Neudoerffer and Lea, 1968).

Growth and Development Influences on Muscle

Composition of muscle tissues are subject to change with growth and development. Robinson (1952) reported an increase of myofibrillar nitrogen with age in breast muscles of chicks from 1 to 4 weeks of age, but noted a decreased percentage of sarcoplasmic and stroma nitrogen in muscles as age advanced. Marion (1965) noted a decrease in the lipid concentrations in the breast muscles of broilers as birds advanced to 63 days of age, while thigh tissues contained a greater quantity of the lipid fraction. Similar results in the muscles of laying hens have been reported (Wangen and Skala, 1968). Fatty acid distribution varies with advancing maturity in the bovine species (Lawrie, 1966) and in maturing pigs (Elson et al., 1963 and

Allen et al., 1967). In poultry muscle, Marion (1965) reported age tends to reduce the level of linoleic acid in the phospholipid fraction, while the level of polyunsaturated fatty acids increased. The phospholipid fraction of broiler muscle tissue was not influenced by age reports the same author, but he noted the quantity of muscle phospholipids decreased as the chickens matured to 63 days. The phospholipid fraction has been reported to vary during the 4-18 days of incubation (Siek and Newburgh, 1965). They evidenced a decline of PC content from 70 to 50 percent with other phospholipids maintaining a consistent level. Marion and Miller (1968) found an increase in percentage of phospholipids in total lipid from 8 to 20 week old chickens.

The degree of finish appears to influence the edible yield of male and female turkeys (Essary et al., 1968), and rations or diets are established factors influencing the degree of finish and/or the quantity of lipid found in turkey carcasses (Neudoerffer and Lea, 1968). The fatty acid distribution of a ration is reported to affect the fatty acid composition of adipose and skin tissues (Mickelberry et al., 1966; Osborn et al., 1969; and Carlson et al., 1969), but rations show little effect on the fatty acid distribution in muscle tissues (Marion and Woodroof, 1966; Chung et al., 1967 and Neudoerffer and Lea, 1968). The position of double bonds in tissue fatty acids is reported to be influenced by the position of the double bond in ration fatty acids (Miller et al., 1969).

Lipid Stability

Factors related to poultry meat stability divide into natural

animal components and technological manipulations. Rate of rancidity development has been shown to be influenced by types of coatings applied to the meat (Marion and Forsythe, 1964), cooking processes (Keskinel et al., 1964), quantity of antioxidant fed (Mecchi et al., 1956). Alteration of the inherent quality of live turkey was suggested by Klose et al. (1952). Fatty acids found in a particular ration have been shown to correspondingly occur in adipose tissue, eggs, heart, liver, oviduct (Machlin et al., 1962), in chicken thigh, breast, and skin tissues (Marion and Woodroof, 1963), and in chicken egg yolk (Sell et al., 1968). The dietary alteration of fatty acids is evidenced in the neutral lipid fraction rather than the phospholipids when examining the adipose tissue of the hen (Issacks et al., 1964), and thigh and breast muscle tissues of the broiler (Marion, 1965). In turkeys, Neudoerffer and Lea (1968) noted changes in neutral lipid fatty acid ratios due to diet, and observed that other fractions were not significantly affected by ration.

Product Dependence on Growth Phenomenon

The distribution of lipid in muscle tissue depends on many factors, and these variations are of interest to food product acceptability and stability. As has been noted, animal variation in lipid content and lipid class content are dependent on specie, sex, muscle class, maturity and diet. These variables direct, in part, the stability and flavor of a product. Total lipid of turkey muscle tissues show a significant relationship with rancidity (Marion and Forsythe, 1964), while Osborn et al. (1969) reported no significant relationship between total

lipid and oxidation rate in tissues obtained from 20, 24, and 28 week old turkeys. Miller et al. (1969) reported double bond position in fatty acids of a ration can be incorporated into tissue fatty acids, and the movement of the double bond to the terminal methyl group of a fatty acid molecule reduces the shelf life of broiler tissues. The presence of tocopherols in muscle tissue plays a major role in establishing the stability of poultry fat (Mecchi et al., 1956). Lipids of male turkey muscles are more susceptible to lipid oxidation when contrasted to lipids of female muscle tissues observed Hartung (1965) and Osborn et al. (1969). Hartung and Froning (1967) reported a significant male and age relationship with less meat stability. Phospholipids have been implicated in off-flavor, off-odor development because they possess the long chain polyunsaturated fatty acids. In muscle, phospholipids function as structural bridges between water-soluble proteins and non-polar lipids (White et al., 1968). During storage, phospholipids are subject to rapid deteriorative change by hydrolysis (Olley and Lovern, 1960; and Fishwick, 1968) and oxidation (Acosta et al., 1966; and Dimick and MacNeil, 1970). Marion (1970) observed no changes in individual phospholipid fractions when male turkey carcasses were stored for 5 months, but he noted the presence of LPC in freshly frozen turkey. Phospholipid rancidity in the turkey has been investigated by Fishwick (1968), and Fishwick and Zmarlicki (1970). Phospholipid concentrations increase in cooked pork and beef products reported Campbell and Turkki (1967), and they evidenced an increase in linoleate concentration of the phospholipids present in cooked pork tissues. Chung et al. (1966)

observed similar fatty acid changes in cooked beef, pork and fish. Hornstein et al. (1967) reported the presence of long chain polyunsaturated fatty acids in the phospholipid fraction of beef muscles. Chang and Watts (1952) reported greater concentrations of polyunsaturated fatty acids occur in poultry tissues than in beef and pork. The presence of polyunsaturated fatty acids in muscle tissue is important due to their potential for deterioration (Lea, 1957). The activity of unsaturated fatty acids increases as one goes from oleic to linolenic reports Hite et al. (1949), and the rates of activity are in a ratio of 1:12:24 respectively. Fatty acids comprise 95 and 75 percent of triglyceride and phospholipid molecules respectively, and they are factors directly related to flavor problems (Younathan and Watts, 1960; Miller et al., 1969). The potential for deterioration expands when one considers the presence of fatty acids containing three or more double bonds, and fatty acids with four, five and six double bonds have been reported in substantial quantities in turkey muscle (Fishwick, 1968). Widmer and Holman (1950) reported greater unsaturation in phospholipid fatty acids and found a relationship with rancidity in rat tissues. Oxidation of phospholipid fatty acids has been reported to be more rapid than the neutral lipid fatty acids in freeze-dried beef (El-Gharbawi, 1964). In the muscle tissue of the turkey, the lipid fraction consisting primarily of fatty acids commonly represents 1-5 percent of the muscle tissue on a wet weight basis (Neudoerffer and Lea, 1968; and Marion, 1970), but this fraction is often responsible for the undesirable, rancid characteristics reducing product acceptability.

In animal tissues, the lipid and fractions of lipid are dependent on certain factors, and the extent to which lipid is variable has an influence on the quality of the product. The mechanisms responsible for these differences have not been completely studied, and it is possible that the machinery necessary to form, use and/or mobilize the lipid is partly responsible for the variations in the variable functions and types of tissues. Because lipid variation and factors related to lipid variation can influence the product, it is important to increase our knowledge of these factors and mechanisms relating to the production of a well accepted, saleable and natural product.

PROCEDURES

Part I. Chronological Age Influences on Lipid Deposition

Muscle samples

The muscle samples used in this study originated from domestic male turkeys. Seventy males of Williams' strain were selected from a flock reared at the Iowa State University Poultry Farm. They were allowed ad libitum access to a commercial starter ration for 8 weeks and a commercial grower ration for the remainder of the experiment (Table 1). After 12 weeks of age, the birds were reared on range.

Table 1. Ration formulae fed to turkeys

Ingredients	Starter ration	Grower ration
Corn	42	50
Soybean meal	42	41
Fish meal	5	-
Dical	2	3
Alfalfa	2	2
Soy oil	2	2
Yeast	2	-
Oyster shell	1.5	1.5
Trace mineral salt	0.5	0.5
P-8 vitamin mix	0.5	0.5

Processing method

At four-week intervals, starting at 4 weeks and ending at 28 weeks, ten turkeys were selected at random for sampling. The turkeys were exsanguinated by severing the jugular vein and carotid artery without previous stunning. Struggling and movement were minimized by placing each turkey in a steel cone. Muscle samples were excised without scalding or feather removal.

Proximate analyses

Muscle samples were freed of skin and adhering lipid deposits and passed through a food grinder with plate perforations of 4 mm. Weighed portions of the ground samples were dried in a 90°C. ± 4° oven for 24 hours. Percentage moisture was calculated on a weight-loss basis. Nitrogen content was determined by the micro-kjeldahl method, and protein content was calculated by the use of the 6.25 factor. Lipid content was determined by weighing a dried aliquot of lipid extract (80°C. ± 2° oven).

Lipid extraction

Samples (20 g.) of ground muscle were twice extracted with cold chloroform-methanol (2:1) by using the procedure of Folch et al. (1957). The two extracts were combined, washed with 0.03M $MgCl_2$, evaporated under reduced pressure, and made to a specified volume. Lipid extraction was accomplished in a cold environment (4°C.).

Phospholipid separation and determination

Separation of individual phospholipids was accomplished by thin-

layer chromatography, utilizing the techniques outlined by Skipski et al. (1965), and the solvent mixture by Parker and Peterson (1965). Filter paper was used to line the inside of the developing tanks and time was allowed for saturation of the environment. Fifty microliters of the total lipid extract were placed on silica gel G plates. Individual phospholipids were identified with the use of standards (Supelco, Inc., Bellefonte, Pa.) and exposure to iodine vapors (Sims and Larose, 1962). The individual phospholipid spots were marked with a spatula, and removed by suction onto a fritted glass disc (Marion, 1970). Each phospholipid was eluted into a test tube, and the solvent was evaporated with a stream of air. Dried phospholipids were digested, and phosphorous was determined using the method by Chen et al. (1956). Phospholipid content was determined by multiplying the phosphorous content by 25 for PC, PE, SPH and PS - PI. LPC content was calculated by using 17 as a factor thus accounting for the loss of its fatty acid. PS - PI were reported together because of inconsistent separation of the two. Total phospholipid content was obtained through the same procedures outlined above.

Fatty acid preparation and determination

Lipid extract portions were saponified and methylated with the use of the BF_3 technique suggested by Luddy et al. (1968) and the Instrumental Techniques Committee (1968). The hexane-soluble fraction was concentrated so that an excess of one microliter was available for analysis. Methyl esters were injected into a F & M Model 810 gas Chromatograph maintaining a hydrogen flame detector temperature of 220°C.

Injector temperature remained at 220°C. while the oven, containing a ¼ x 6 ft. stainless steel column packed with 15% diethylene glycol succinate on Chromosorb W (AW), was operated in a temperature range of 180-205°C. Identification of fatty acids was accomplished by comparing the retention times of methyl ester preparations with known standards (Supelco, Inc., Bellefonte, Pa.). A quantitative estimate of the relative distribution of each fatty acid was accomplished with the use of an Infotronics Electronic Digital Integrator (CRS-100A).

Part II. Investigation Related To Lipid Accumulation

Muscle samples

The muscle samples used in this experiment were obtained from domestic male turkeys purchased from a commercial grower. Forty Nicholas males were raised at the Iowa State University Poultry Farm for the duration of the experiment. They were allowed ad libitum access to commercial rations and grown in confinement.

Processing method

Beginning at twelve weeks and ending at 24 weeks, 8 turkeys were randomly selected at 4-week intervals for processing. The turkeys were exsanguinated by severing the jugular vein and carotid artery without previous stunning. Immediately after severing the vein and artery, muscle samples from the Pectoralis major, Biceps femoris, Gastrocnemius and wing muscles were excised and prepared for histological examination. The muscle strips were placed in liquid nitrogen before 5 minutes of exsanguination had elapsed. The same muscles from the opposite side

of the carcass were excised, frozen and kept for additional analyses.

Proximate analysis

Muscle samples were frozen in liquid nitrogen and pulverized with the use of a Sorval Omni-Mixer. The resulting powder was used for moisture, lipid and protein determinations as described in Part I. Total phospholipids were determined as outlined in Part I.

Histological techniques

Fresh muscle tissue was cut with a razor blade to an approximate size for histological examination. Care was taken so that the blade touched no more than one muscle of any bird. After freezing, the samples, along with some ice flakes to prevent dehydration, were placed in a pre-cooled (liquid N_2) plastic bag, and held in the freezer (-30°C.) until analyses could be run. Samples were mounted on crystal posts and sectioned at 20 μ in a -20°C. cryostat. Sections were transferred to a pre-cooled watch glass containing 2-3 drops of pre-cooled incubation media. The sections froze upon contact, but thawed readily when incubation started. Incubation for the enzymes ranged from 15 minutes to one hour at 37°C. in a moistened atmosphere. Incubation media for enzymes were prepared after the techniques of Barka and Anderson (1963), but only 5 mg/ml of 6-phosphogluconate was prepared because it represents a saturated solution (Nachlas et al., 1958). A 0.1M phosphate buffer adjusted to pH 7.0 was used and 0.1M cyanide inhibitor was included in the incubating media. Enzymes studied in this experiment included DL-hydroxybutyric dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and lactate dehydrogenase.

To verify the validity of incubating media, pork muscle was used as a control. Results are reported as percentages of enzyme-positive fibers.

Enzyme analyses

Approximately 3-5 gms. of muscle tissue were used to prepare mitochondria according to the procedure by Lundquist and Kiessling (1967). A tris-phosphate buffer (pH 7.5) was mixed with the muscle tissue in a Potter-Elvehjem homogenizer (Kontes). The mitochondria were obtained by differential centrifugation (10 minutes at 1000 G and at 10,000 G for 20 minutes saving precipitate at 5°C.). Muscle mitochondria were frozen to lyse the walls, and then incubated in a Biological Oxygen Monitor at 37°C. One cell contained a fraction of mitochondria plus buffer as a control and the other cell contained the buffer, mitochondria and substrate (DL hydroxybutyrate). Oxygen consumption (microliters) was measured.

About 3 grams of muscle tissue was minced in a Potter Elvehjem homogenizer with 0.25M sucrose. The contents were centrifuged at 1000 x G for 15 minutes to remove unbroken cells and other fragments and the supernatant was used to determine the activity of lactate dehydrogenase and glucose-6-phosphate of lactate dehydrogenase. Glucose-6-phosphate dehydrogenase activity was determined on the Biological Oxygen Monitor at 37°C. using the reaction medium described by Dawson and Romanul (1964). The reaction mixture used for lactate dehydrogenase was obtained from the same source, but its activity was measured on a Beckman DU Spectrophotometer at 340 nanometers and at a temperature of 26°C. The amount of protein was determined by using the Biuret

technique (Torten and Whitaker, 1964).

Statistical analysis

Least squares analyses were used to estimate age, muscle type, and other effects according to the procedures of Snedecor and Cochran (1967). Regression and correlation values were calculated by procedures of the same authors. Duncan's Multiple Range Test was used by the procedure outlined by Steel and Torrie (1960).

RESULTS AND DISCUSSION

The results and discussion of the compositional changes in the tissues of maturing turkeys are presented in two parts. In the first part, thigh muscle tissue involved the use of all muscles in that area, meaning that inter- and intramuscular variability is included in these data. Breast muscle data represent intramuscular variability only. In part 2, intramuscular variability is represented in the Pectoralis major, Biceps femoris, and Gastrocnemius muscles which represent the breast, thigh and leg respectively. In the wing tissues however, all muscles in the first joint were used because of their small, individual size, and inter- and intramuscular variability exists in these data.

Part I

The average weights of the turkeys used in this experiment are presented in Table 2. These data indicate the turkeys weighed less than normally would be expected for turkeys of their ages. At 20 weeks of age, turkeys will more commonly weigh 10-11 Kg. live weight.

Proximate analysis

Table 3 and Figure 1 present the average moisture percentages of breast and thigh tissues. Within the age groups, moisture percentages in breast tissues were consistently lower than those in thigh tissues. Breast is commonly believed to be the drier of the two tissues. Moisture levels were significantly related to age and type of tissue as shown by analysis of variance (Table 34, Appendix). Table 4 shows a negative relationship between moisture and age and the extent of that

Table 2. Weight of tom turkeys at various ages

Age (weeks)	Weight (Kg.)	Standard Error
4	1.134[a]	0.11
8	1.950	0.23
12	4.024	0.54
16	6.365	0.51
20	7.690	1.11
24	10.612	1.38
28	11.543	1.63

[a] Each mean is based on 10 observations

Table 3. Moisture levels in breast and thigh tissues at various ages

	Weeks						
Tissue	4	8	12	16	20	24	28
Breast	75.26[c]	74.65[cd]	72.74[f]	73.45[ef]	73.37[ef]	73.16	72.72[f]
Thigh	78.31[a]	77.83[a]	76.38[b]	76.43[b]	76.17[b]	75.07[c]	74.12[de]

[c] Means with the same superscript are not significantly different at 0.01 level

association. Thigh moisture levels decrease at a faster rate with increasing turkey age. Thigh lipid and breast and thigh protein increase with age. Thigh tissues consistently contained lower concentrations of protein (Table 5 and Figure 2). In both tissues, protein concentration significantly increased with age, and its content is significantly dependent on type of muscle tissue. Robinson (1952) reported that

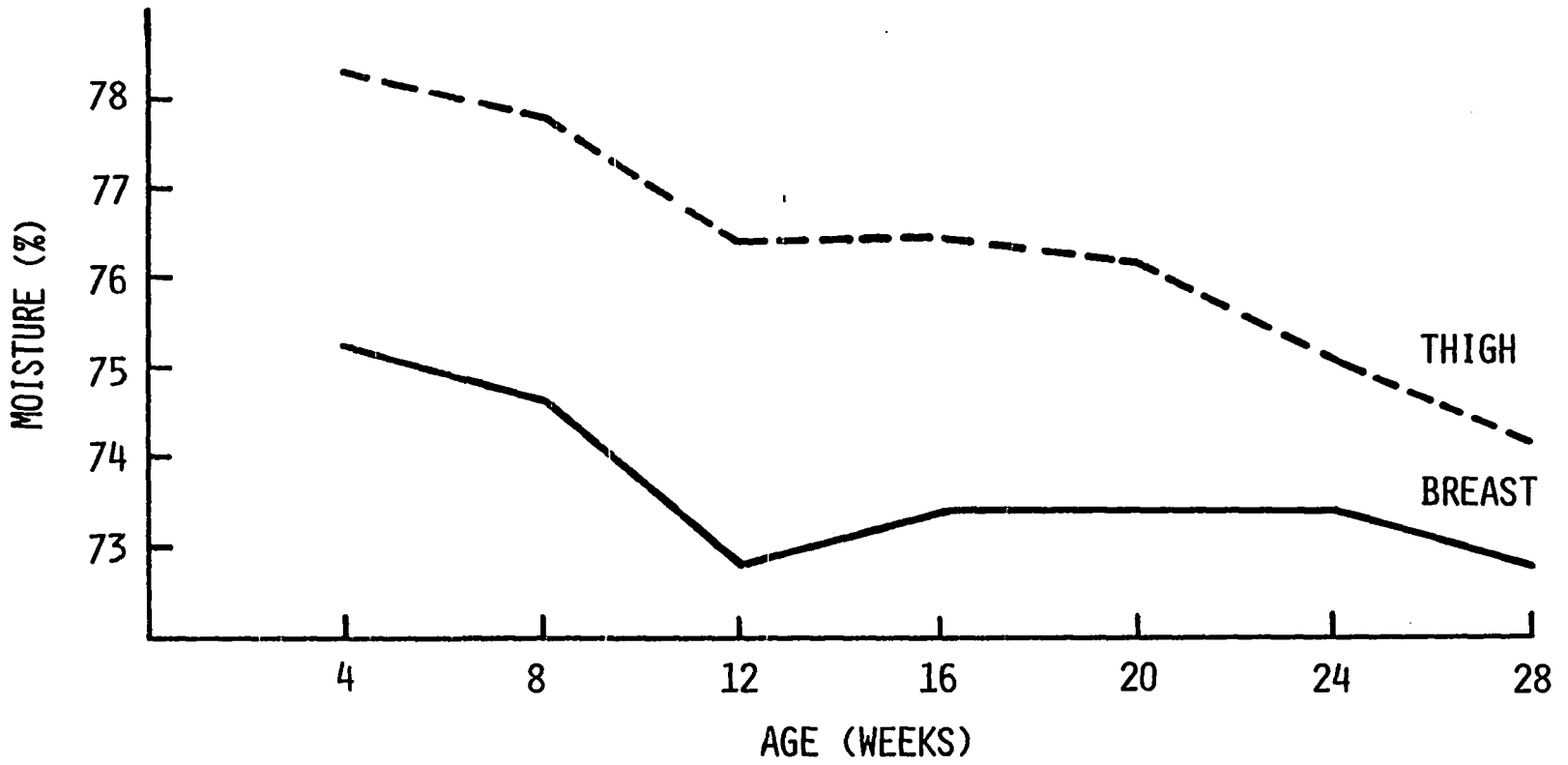

Figure 1. Percent moisture of Pectoralis major and thigh tissues at various ages

Table 4. Relationship of proximate analysis of breast and thigh with age

Factor	Tissue	Regression coefficient
Moisture	Breast	-0.356*
	Thigh	-6.536**
Lipid	Breast	0.017
	Thigh	0.311**
Protein	Breast	0.362**
	Thigh	0.292**

* Significant at 0.05 level

** Significant at 0.01 level

Table 5. Protein concentration in tissues at various ages

Tissue	Weeks 4	8	12	16	20	24	28
Breast	22.63[b]	23.57[b]	25.33[a]	25.36[a]	25.44[a]	25.19[a]	24.88[a]
Thigh	18.70[c]	19.89[c]	20.89[c]	21.49[c]	20.77[c]	21.01[c]	20.72[c]

[b] Means with same superscript are not significantly different at 0.01 level within the whole table

protein content increased as the chick advanced in age.

The lipid concentration is significantly dependent on age of the turkey and type of muscle tissue (Table 34, Appendix). In Table 6 and Figure 3, it is evident that the thigh tissues contain greater quantities of lipid than breast at all ages. Acosta et al. (1966) and Neudoerffer and Lea (1968) reported similar findings. Although the lipid content is dependent on the type of muscle tissue, regression coefficients in Table 4 show significant trends in the age-lipid content

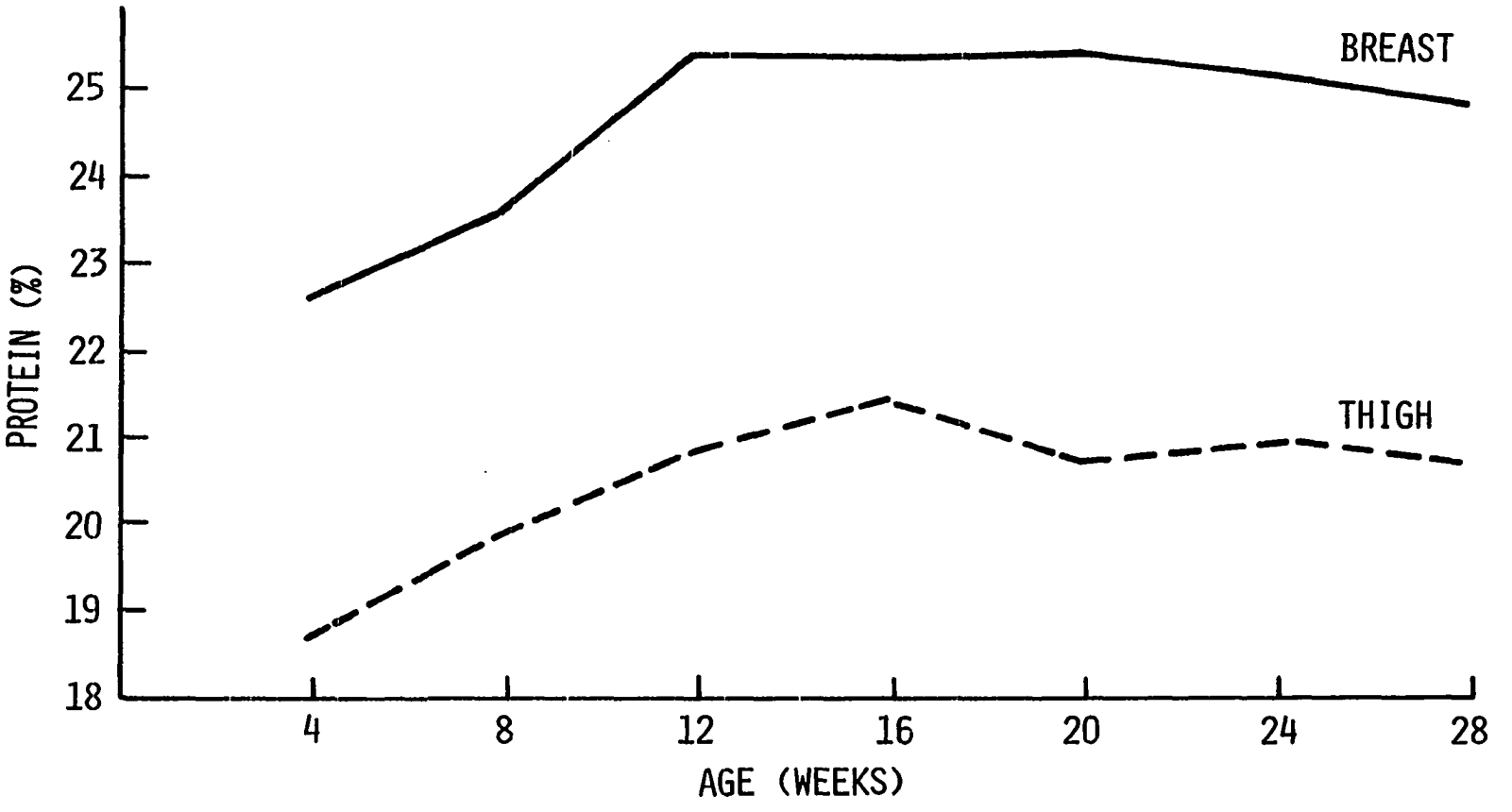

Figure 2. Percent protein of Pectoralis major and thigh tissues at various ages

Table 6. Lipid content in breast and thigh tissues at various ages

Tissue	Age (weeks) 4	8	12	16	20	24	28
Breast	1.00[fg]	0.79[g]	0.79[g]	0.90[fg]	0.73[g]	0.96[fg]	1.07[efg]
Thigh	2.28[bc]	1.66[cde]	1.86[bcd]	1.49[def]	2.48[b]	3.53[a]	3.73[a]

[f] Means with the same superscript are not significantly different at 0.01 level

relationships. Lipid content of breast muscle tissues was consistent throughout these ages. Marion (1965) reported a significant decrease in breast lipid content as broilers advanced in age, but no association was elucidated for thigh tissues. Turkey thigh tissues, however, show a highly significant, positive relationship with age. The lipid content to increase significantly after 16 weeks of age as noted by the thigh lipid content means. Marion and Forsythe (1964) reported total lipid is directly associated with the uptake of oxygen, thus accentuating the importance of more complete information on lipid deposition in muscle tissues.

Phospholipids in tissues

Quantitative phospholipid distribution in muscle tissue was studied as the turkey advanced toward market age. The relatively high degree of unsaturation of phospholipid fatty acids (Fishwick, 1968) suggests this group of lipids is involved in the ultimate formation of compounds which impart off-flavors (Lea, 1957). The relationship of lipid composition and rate of oxidative deterioration of turkey reported by Keskinel et al.

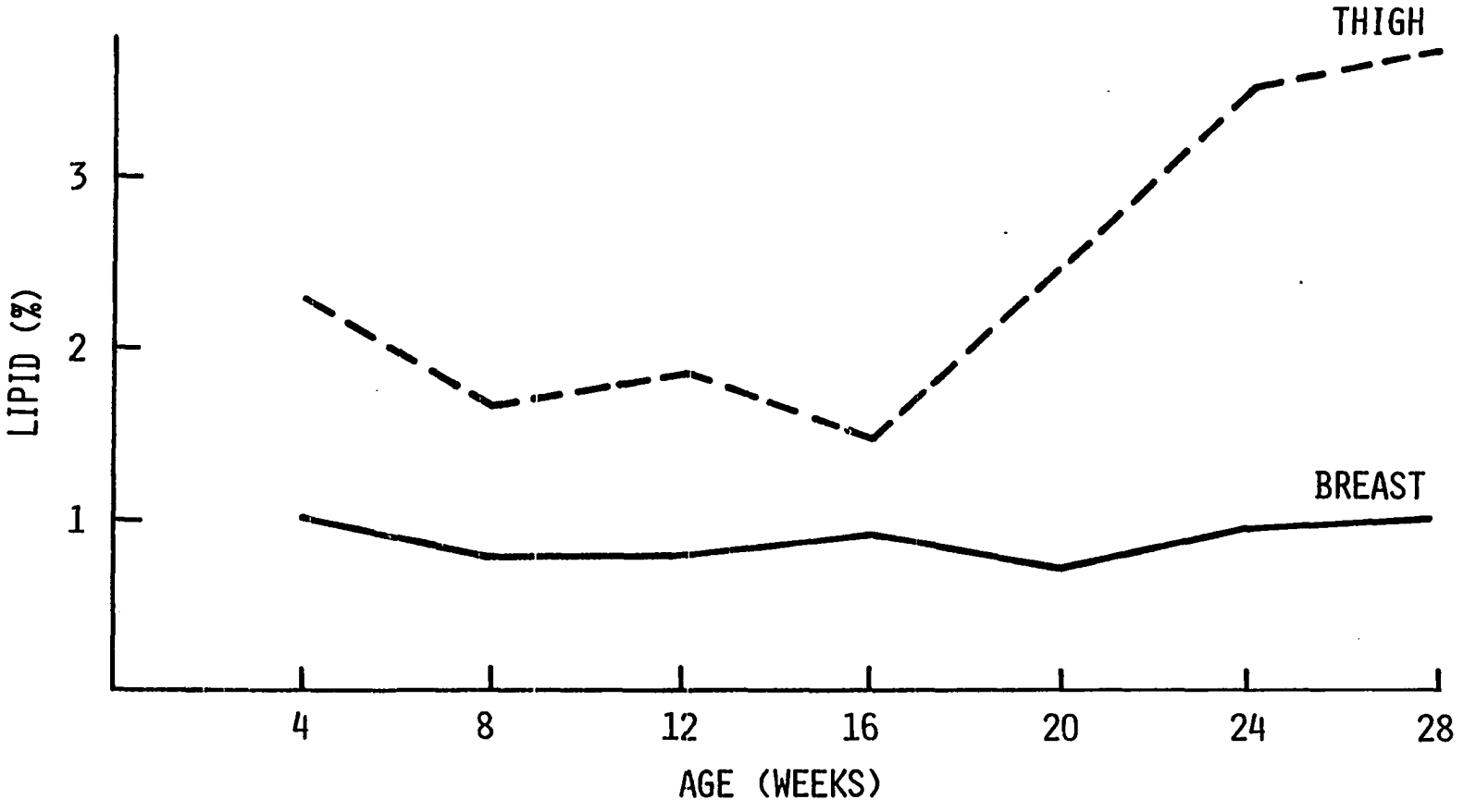

Figure 3. Percent lipid of Pectoralis major and thigh tissues at various ages

(1964), and Marion and Forsythe (1964) is important.

The distribution of phospholipids in muscle tissues is presented in Table 7. PI and PS appeared as a single spot because of incomplete

Table 7. Phospholipid distribution in turkey breast and thigh tissues

Phospholipid	Breast			Thigh		
	Mg/100g. Fresh Tissue		Percent	Mg/100g. Fresh Tissue		Percent
PE	125.28	.015[a]	22.84	189.96	.015	27.71
PS PI	54.10	.003	9.81	57.88	.003	8.41
PC	280.65	.028	51.90	338.85	.028	50.14
SPH	57.49	.006	10.42	65.80	.006	9.66
LPC	27.69	.001	5.00	27.80	.001	4.05

[a] Standard error of the mean

separation of these phospholipids. All major phospholipids were detected in both tissues at each age period. LPC, though present in small quantities, appeared in all samples, thus supporting the suggestions by Marion (1970) that LPC is a normal constituent of muscle lipids, and the data reported here support this suggestion. PC represented approximately half the phospholipids in both tissues at all age groups, and therefore is the phospholipid in greastest abundance. Similar distribution of phospholipids in turkey muscle tissues was reported by Neudoerffer and Lea (1968) and Marion (1970). PE is reported as the second largest phospholipid in both tissues. Moreover, it appears to differ between muscle tissues. Because of this difference the "cephalin" fraction (PE, PS, PI) accounts for a larger portion of the phospholipids

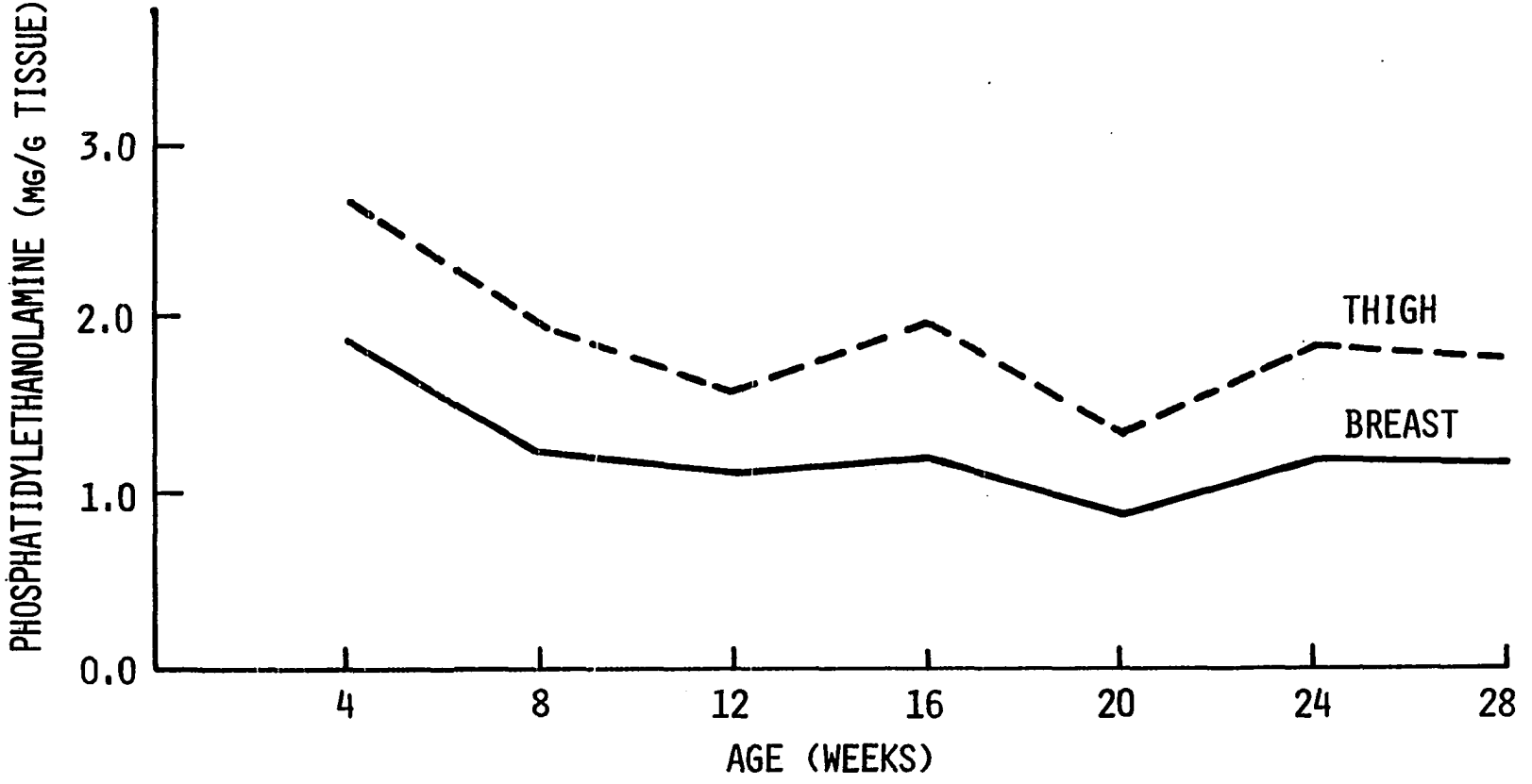

Figure 4. Phosphatidylethanolamine content of Pectoralis major and thigh tissues at various ages

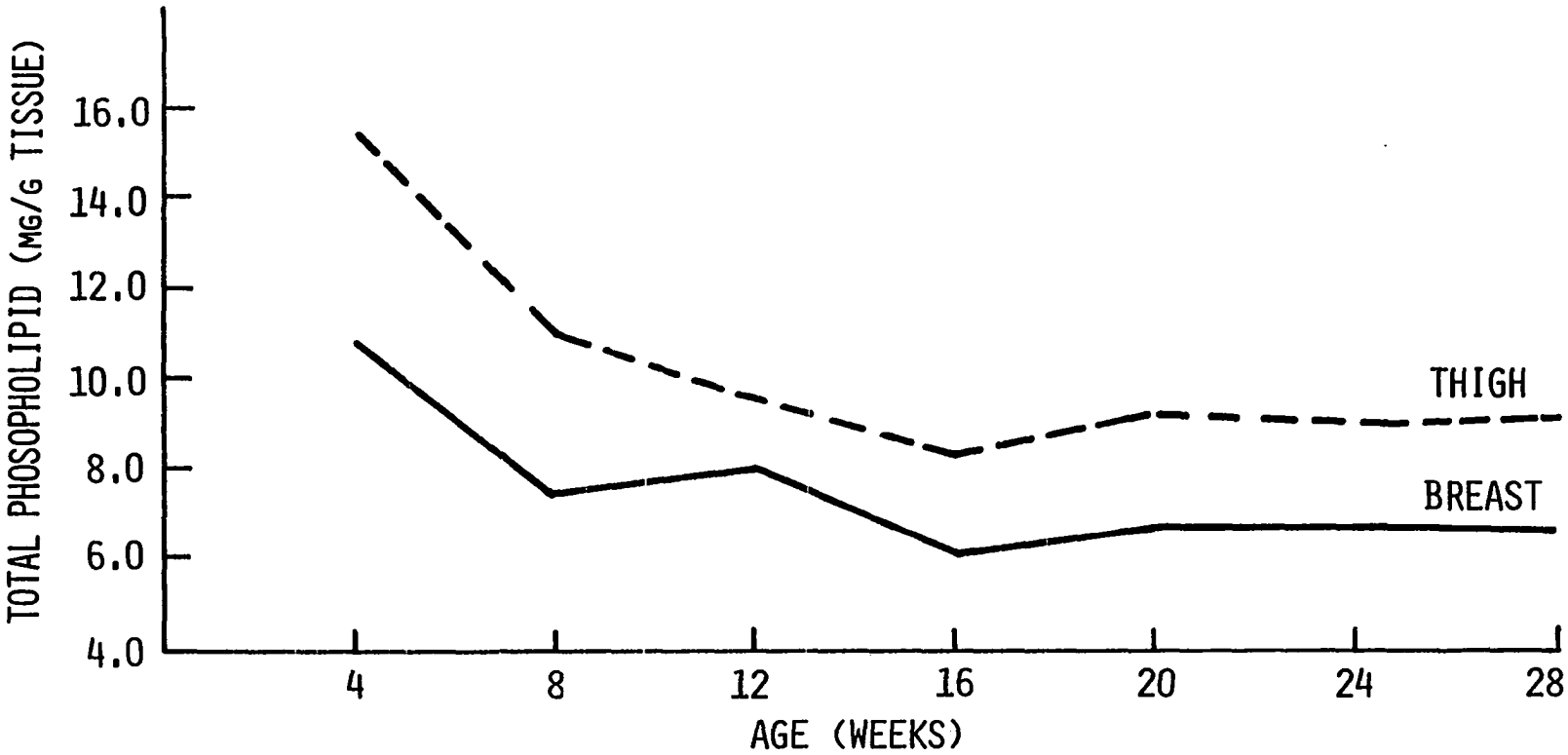

Figure 5. Phospholipid, mg per gm tissue, from Pectoralis major and thigh tissues at various ages

in thigh muscle than in breast muscle. Peng and Dugan, (1965) and Acosta et al. (1966) reported similar evidence in chicken and turkey tissues, respectively.

Table 8 presents the average phospholipid content of each muscle tissue at the various ages. As shown in Table 7, thigh tissues contained greater quantities of phospholipid than breast tissues. Within each age group, phospholipid content was greater in thigh tissues. The analysis of variance (Table 35, Appendix) shows this significance of muscle type on the distribution of the phospholipids. Only LPC was non-significantly dependent on muscle type. PS - PI was significant at the 0.05 probability level, but all others appear highly significant with muscle type. In each tissue, the highest proportion of phospholipid occurred at 4 weeks of age. All phospholipids were significantly related to age, but the relationships were non-linear (Table 9) when values were expressed as mg phospholipids per gram of tissue. When phospholipids were expressed as a percentage of the total lipid, a significant and negative regression coefficient existed between age and PE levels for both tissues (Figure 4), suggesting a decrease in this major component even though total lipid of breast remained constant with increasing age. Total phospholipid, expressed in the same manner, was significantly affected by age and muscle type (Table 36, Appendix). A negative and significant linear regression coefficient (Table 9) existed between age and total phospholipids in both muscle tissues. The linear regression coefficient for thigh was 1.5 times greater than that of the breast. The proportions of phospholipid to total lipid are presented in

Table 8. Phospholipid content of breast and thigh muscles of turkeys at various ages

Muscle	Phospholipid (mg/100g Fresh tissue)	Age (Weeks) 4	8	12	16	20	24	28
Breast	PE	186.68	123.89	113.85	120.75	92.16	120.33	119.30
	PS	72.72	57.95	48.49	44.92	33.47	59.65	61.51
	PC	334.27	291.95	251.53	299.85	231.02	287.44	268.46
	SP	72.89	63.91	40.75	44.55	42.61	70.28	67.42
	LPC	34.49	29.39	18.68	19.18	18.62	40.48	32.97
	Total	701.05	567.09	473.30	529.26	417.88	578.18	549.66
Thigh	PE	268.66	197.39	161.22	199.09	137.17	186.31	179.90
	PS	74.50	67.96	45.71	51.87	34.50	64.28	66.31
	PC	441.60	333.67	273.20	359.54	286.48	347.67	333.82
	SP	91.29	65.44	48.53	45.41	53.24	80.21	76.48
	LPC	33.73	29.96	19.38	18.71	18.96	37.52	36.32
	Total	909.28	694.42	548.04	674.63	526.34	715.98	692.82

Table 9. The regression coefficients of various parameters to age of turkey

Parameters	Breast		Thigh	
	b_1[a]	b_{11}	b_1	b_{11}
Phospholipids (mg/g tissue)				
PC	-.081	.044	-.102	.092
PE	-.468	.153	-.045	.061
PS - PI	-.162	.029*	-.154	.305*
Sph	.001	.033*	-.004	.042*
LPC	.006	.018	.008	.019*
Total Phospholipids	-1.741	1.742	-2.243	2.487
Phospholipids (% of total lipid)				
PC	.135	.852**	.176	.511
PE	-.706*	.135	-.709*	.084
PS - PI	.001	.239	.017	.176
Sph	.312	.292	.278	.270
LPC	.258	.185	.238	.149
Total Phospholipids	-2.681*	.252	-4.191*	.473

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

Table 10 and Figure 5. Phospholipids appear as the major class of lipids from both tissues at 4 weeks of age, but decline with increasing age of the turkey. Moreover, within each age group, the phospholipid fraction of breast tissues is significantly greater than the phospholipid fraction of thigh tissues.

Table 10. Percent total phospholipid of total lipid in breast and thigh muscles of turkeys at various ages

Tissue	Age (weeks) 4	8	12	16	20	24	28
Breast	100.00[a]	95.03[a]	100.00[a]	68.60[c]	93.09[ab]	71.01[bc]	61.99[cd]
Thigh	68.06[a]	66.71[a]	51.88[d]	57.29[d]	37.71[de]	25.87[e]	24.64[e]

[a] Means with the same superscript are not significantly different at 0.05 level

The data suggest that some phospholipids "plateau" during the age periods studied. Plateauing was observed by the time the turkeys were 8 weeks of age. This trend occurred with all phospholipids even though not all were significantly related to the age of the turkey. This consistent proportion of phospholipids probably has little effect on the variation others have observed in rancidity development. Variation of that type is due, at least in part, to differences such as among turkeys and among muscle classes. Variability in degradation products reported by Fishwick and Zmarlicki (1970) and Dimick and MacNeil (1970) appears to be related to the quantity of unsaturated fatty acids rather than merely their association with the phospholipid class of lipids, al-

though these two factors are related.

Lipids not only varied in content between the two muscles, the rate of lipid accumulation in thigh tissues increased after 16 weeks of age (Table 6). On an absolute basis, thigh contained larger quantities of phospholipid. However, phospholipids comprised a larger percentage in breast lipid. A similar trend was reported by Marion and Miller (1968) for chicken breast and thigh tissues. In beef tissues, Hornstein et al. (1967) reported the lipid content was not followed by phospholipids, but by neutral lipids. These findings suggest that the phospholipid class is associated with the physiological activity of muscle since the thigh muscles of the domestic turkey, which contain higher quantities of phospholipids, are more active than the breast muscle.

The Biceps femoris muscle was excised from thighs of the alternate half of the turkeys for analysis. Because of the small size of this muscle at earlier ages, only 20, 24, and 28 week old birds were sampled. Proximate analysis and phospholipid data of the Biceps femoris muscle at various ages are presented in Table 11. A greater concentration of lipid appeared in Biceps femoris muscle than in breast tissue, but was lower than that of thigh tissue in general. Biceps femoris lipid content was significantly affected by age (Table 37, Appendix). Moreover, Table 12 presents regression coefficients showing the positive and significant age effect. Biceps femoris lipid content and thigh lipid content increased at a differing rate as age advanced. Lipid content of Biceps femoris and thigh were not similar to breast lipid content.

Table 11. Mean composition of Biceps femoris from turkeys of various ages

Factor	Age (weeks) 20	24	28
Lipid (%)	1.30	1.92	2.18
Protein (%)	21.06	20.97	20.77
Moisture (%)	76.62	75.79	75.48
Phospholipid (mg/100 g tissue)	520.10	701.10	693.20
Phospholipid (% of total lipid)	41.00	37.49	32.70

Table 12. Relationship of Biceps femoris variables with age

Factor	Regression coefficients b_1[a]	b_{11}
Lipid (%)	0.111**	-0.011**
Protein (%)	-0.050	0.006
Moisture (%)	-0.142*	0.016
Phospholipid (mg/100 g meat)	0.216	-0.054**
Phospholipid (% of total lipid)	-1.038*	-0.040

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

Percent moisture and percent protein were not affected by age. Proximate analysis values of Biceps femoris approximate corresponding data of the thigh rather than breast data (Tables 3, 5, and 6). Phospholipids, both percent of lipid and milligrams per gram of tissue, appear similar to thigh data. Percent phospholipid and phospholipid values are significantly realted with age. However, the phosphlipid values show a positive relationship with age, while percent phospholipid shows a negative relationship. These results differ from those of thigh and breast, suggesting variable metabolic and physiologic needs of muscle tissues.

Table 13. Cholesterol plus cholesterol ester levels in muscle tissues from turkeys of various ages

Ages (Weeks)	Breast		Thigh	
	Mg/100 gms Tissue	Percent of Total Lipid	Mg/100 gms Tissue	Percent of Total Lipid
8	90.88^{e}	11.50^{tu}	156.67^{abcd}	9.43^{u}
12	92.27^{e}	11.70^{tu}	131.84^{d}	7.09^{v}
16	140.31^{cd}	15.90^{s}	178.33^{ab}	11.98^{t}
20	84.24^{e}	11.51^{tu}	155.87^{bcd}	6.28^{vw}
24	97.00^{e}	10.10^{tu}	185.60^{a}	5.25^{vw}
28	70.37^{e}	6.55^{vw}	166.99^{abc}	4.47^{w}

[a] Means with the same superscript are not significantly different at 0.01 level

The cholesterol - cholesterol ester data on turkey are presented in Table 13. Cholesterol levels were significantly affected by age and muscle type (Table 38, Appendix). Between muscles, thigh cholesterol

levels were higher at each period as determined by Duncan's multiple range test. The significant interaction of age and muscle type may be due to the general tendancy for breast cholesterol levels to decline through these age periods while thigh cholesterol quantities generally increase. Cholesterol, expressed as a percent of total lipid, decreases significantly after 16 weeks of age in both tissues (Table 13). Moreover, both tissues show highest concentrations at 16 weeks of age. The cholesterol levels presented in Table 13 are slightly higher than cholesterol levels of the 10-week-old turkeys reported by Neudoerffer and Lea (1968), and slightly higher than cholesterol levels of 8-week-old broilers reported by Mickelberry et al. (1966) and Marion and Woodruff (1965). Variation noted between muscle type and age with cholesterol plus cholesterol esters suggest variable lipid requirements of tissues.

Table 14. Regression coefficients of cholesterol plus cholesterol esters with age

Factor	b_1[a]	b_{11}
Percent cholesterol		
Breast	-.234**	-.045**
Thigh	-.318***	-.044***
Cholesterol		
Breast	-1.032*	-.314**
Thigh	1.360	-.040

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

Fatty acids

The distribution of fatty acids in breast and thigh tissues is presented in Table 15. Twenty-four fatty acids are presented in this table, however numerous peaks appeared on the chromatogram that could not be satisfactorily identified because of low concentration and/or inconsistent appearance. Approximately 83 percent of all fatty acids found in both turkey tissues were represented by acids of carbon length double bond number 15:0, 18:0, 18:1, 18:2, 20:4 and 26:0. Of these major fatty acids, breast muscle, in comparison with thigh, contained significantly greater concentrations of pentadecanoic (15:0), arachidonic (20:4) and cerotic (26:0) fatty acids. The opposite tissue effect was observed for linoleic acid (18:2). Linolenic acid (18:3) was not detected at 24 and 28 weeks of age in either tissue. The individual fatty acids at each age period are presented in Table 16, and their relationships with age are presented in Table 17 and Table 18. A significant effect of age was observed on all fatty acids. Muscle type showed a significant effect on all fatty acids except for caprylic (8:0) and palmitic (16:0). Approximately half of the fatty acids studied had a positive relationship with age. Of the most abundant fatty acids, oleic (18:1) increased significantly with age in both tissues while palmitic (16:0) significantly increased in thigh tissues. Pentadecanoic (15:0), oleic (18:1), and arachidic (20:0) significantly increased with age in breast tissues. The fatty acid changes suggest variable metabolism in muscles throughout these age periods. Schuler and Essary (1971) suggested that variable metabolism accounted for variation in fatty acid

Table 15. Distribution of fatty acids in turkey breast and thigh

Fatty Acid[a]		Breast (%)	Thigh (%)
8:0	Caprylic	0.14	0.09
9:0	Nonanoic	0.26	0.11
10:0	Capric	0.20	0.06
11:0	Undecanoic	0.23	0.14
12:0	Lauric	0.37	0.20
13:0	Tridecanoic	0.42	0.17
14:0	Myristic	0.56	0.43
15:0	Pentadecanoic	5.22*[b]	2.24
16:0	Palmitic	15.79	15.75
16:1	Palmitoleic	1.59	2.00
17:0	Heptadecanoic	1.49	0.85
18:0	Stearic	12.58	11.27
18:1	Oleic	14.57	17.97
18:2	Linoleic	22.16	30.02*
18:3[d]	Linolenic	0.56	1.89**
20:0	Arachidic	1.11	1.59
20:1	Eicosenoic	0.77	0.44
20:4	Arachidonic	1.11	1.59
22:0	Benenic	0.49	0.27
22:1	Erucic	0.47	0.24
22:5[c]		1.35	0.63
22:6[c]		1.27	0.64
24:0	Lignocaric	1.02	0.48
26:0	Cerotic	4.58**	1.93

[a] Carbon chain length: number of double bonds

[b] Statistical significance between means of two tissues by Duncan's Multiple Range Test, * at 0.05 level, ** at 0.01 level

[c] Tentative identification

[d] Means based on five age periods

Table 16. Mean (%) fatty acids in breast and thigh muscles of turkeys of various ages

	Age (weeks)							
	4		8		12		16	
Fatty Acid	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
8:0	-	-	-	-	-	-	-	-
9:0	0.22	0.08	0.24	0.09	0.36	0.24	0.36	0.12
10:0	0.17	0.06	0.14	0.03	0.30	-	0.31	0.12
11:0	0.13	0.05	0.20	0.08	0.25	0.48	0.16	0.13
12:0	0.25	0.08	0.27	0.15	0.28	0.12	0.54	0.21
13:0	0.29	0.13	0.39	0.14	0.53	0.25	0.74	0.23
14:0	0.71	0.09	0.37	0.14	0.57	0.31	0.28	0.13
15:0	2.15	2.14	5.35	2.49	5.87	2.23	6.24	3.45
16:0	16.95	13.75	12.22	10.36	15.13	15.60	15.32	14.16
16:1	1.20	2.01	0.92	1.12	1.57	1.53	1.60	1.30
17:0	0.98	0.93	1.75	0.99	1.94	0.73	2.27	1.25
18:0	14.13	11.00	12.65	9.51	13.77	13.65	12.82	12.79
18:1	11.06	17.64	10.34	13.50	13.50	15.91	15.10	12.84
18:2	25.69	32.97	20.62	26.28	22.57	32.28	21.30	26.61
20:0	0.46	1.28	0.56	0.86	0.53	0.71	1.25	0.40
20:1	0.79	0.41	0.66	0.41	0.92	0.63	0.86	0.52
20:4	6.43	4.85	8.49	4.81	8.99	5.75	8.00	6.52
22:0	1.42	0.53	0.39	0.36	0.46	0.24	0.42	0.22
22:1	0.55	0.22	0.35	0.12	0.42	0.43	0.19	0.16
22:5[a]	1.23	0.67	1.56	0.80	1.11	0.57	1.84	0.99
22:6[a]	0.89	0.58	1.20	0.54	1.16	0.63	1.03	0.40
24:0	7.70	3.27	5.47	2.51	2.90	1.38	2.44	1.58
26:0	1.01	0.57	1.07	0.64	1.36	0.74	1.40	1.07

[a] Tentative identification

Table 16. Continued

Fatty acid	20		24		28	
	Breast	Thigh	Breast	Thigh	Breast	Thigh
8:0	0.21	0.10	0.03	0.09	0.19	0.08
9:0	0.26	0.11	0.13	0.07	0.22	0.09
10:0	0.22	0.04	0.12	0.05	0.13	0.09
11:0	0.59	0.11	0.12	0.05	0.13	0.05
12:0	0.39	0.18	0.22	0.10	0.63	0.64
13:0	0.44	0.24	0.14	0.05	0.42	0.18
14:0	0.53	0.63	0.52	0.62	1.03	1.09
15:0	6.20	2.27	5.63	1.57	5.09	1.52
16:0	15.03	18.07	16.63	19.61	15.75	18.71
16:1	1.59	2.01	1.98	2.87	2.26	3.18
17:0	2.48	0.97	0.45	0.49	0.56	0.58
18:0	12.17	11.37	11.35	10.38	11.00	10.18
18:1	15.11	19.64	18.41	23.04	18.46	23.25
18:2	21.05	31.28	22.67	31.03	21.20	30.00
20:0	1.58	2.54	1.71	2.73	1.70	2.62
20:1	0.71	0.53	0.56	0.27	0.88	0.31
20:4	9.30	4.51	7.93	3.06	7.70	2.86
22:0	0.36	0.25	0.36	0.08	0.27	0.19
22:1	0.98	0.37	0.31	0.23	0.50	0.14
22:5	1.47	0.72	0.86	0.30	1.37	0.33
22.6	1.16	0.44	0.83	0.33	0.88	0.46
24:0	3.52	2.33	4.91	1.39	5.16	1.04
26:0	1.28	0.48	1.53	0.44	1.23	0.52

Table 17. The relationship of fatty acids to age of turkey, based on the use of orthogonal polynomials

Fatty Acid	Breast		Thigh	
	b_1[a]	b_{11}	b_1	b_{11}
8:0	0.03**	0.01	0.02**	0.00
9:0	-0.01	-0.01	-0.01	-0.01
10:0	-0.01	-0.02**	0.01	0.00
11:0	0.01	-0.02*	-0.02	-0.02**
12:0	0.04*	0.01	0.06**	0.02**
13:0	-0.01	-0.03*	-0.01	-0.01
14:0	0.04	0.05**	0.15**	0.03**
15:0	0.31**	-0.30**	-0.17**	-0.12**
16:0	0.07	0.14*	1.08**	-0.02
16:1	0.18**	0.02	0.24**	0.11**
17:0	-0.13**	-0.17**	-0.08**	0.04**
18:0	-0.58**	-0.04	-0.28**	-0.30**
18:1	1.34**	0.02	1.18**	0.47**
18:2	-0.56**	0.02**	-0.48**	0.06
20:0	0.25**	-0.01	0.33**	0.09**
20:1	-0.01	0.00	-0.03**	-0.03**
20:4	0.05	-0.19**	-0.46**	-0.24**
22:0	0.14**	0.05**	-0.06**	0.01**
22:1	0.01	0.01	-0.01	-0.02*
22:5	-0.03	-0.02	-0.08**	-0.04**
22:6	-0.04	-0.03*	-0.04**	0.00
24:0	-0.33**	0.42**	-0.32**	0.04
26:0	0.04*	-0.03*	-0.04*	-0.03**

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

distribution in broiler tissues. In the thigh tissues, myristic (14:0), palmitic (16:0), palmitoleic (16:1), and oleic (18:1) significantly increased with increasing turkey age. Fishwick (1968) reported these fatty acids are commonly found in the neutral lipid fraction. Hornstein et al. (1967) observed neutral lipid changes closely correlated with total lipids in beef muscle. The negative association of the long-chain polyunsaturated fatty acids with turkey age, however, suggests the decreased relative importance of phospholipids (Fishwick, 1968).

From the analysis of variance (Table 39, Appendix) all fatty acids studied were affected by muscle type with the exception of caprylic (8:0) and palmitic (16:0). In Table 15, linoleic acid content of the thigh is significantly greater than breast linoleic content. Within each age period, linoleic content appeared in significantly greater quantities in thigh tissues in the 4, 12, 20, 24 and 28 week periods (Table 18). Oleic acid responds in a similar manner (Table 39, Appendix), however, oleic shows a closer association with age. These major fatty acids show significant interactions (Table 39, Appendix), again suggesting some variability in the physiological need for lipid. Oleic and linoleic, common fatty acids of neutral lipids (Fishwick, 1968), appear in greater quantities in the more physiologically active thigh tissues in the age period when changes occur. Alterations within lipid fractions occur because of lipid content changes throughout age and these changes may be explained in part by changing energy needs for growth and/or body insulation as necessary. These fatty acid changes follow the lipid content responses in both tissues. Cerotic (26:0) acid showed a

Table 18. Tissue fatty acids (%) at different ages

Fatty Acid	Age (Weeks) 4	8	12	16	20	24	28
Pentadocanoic (15:0)							
Breast	2.2^{bc}	5.4^{a}	5.9^{a}	6.2^{a}	6.2^{c}	5.6^{a}	5.1^{a}
Thigh	2.1^{bc}	2.5^{bc}	2.2^{bc}	3.5^{b}	3.5^{b}	1.6^{c}	1.5^{c}
Oleic (18:1)							
Breast	11.1^{f}	10.3^{f}	13.5^{ef}	15.1^{de}	15.1^{de}	18.4^{cd}	18.5^{cd}
Thigh	17.6^{cd}	13.5^{ef}	15.9^{cde}	12.8^{ef}	19.6^{bc}	23.0^{ab}	23.3^{a}
Linoleic (18.2)							
Breast	25.7^{bc}	20.6^{c}	22.6^{c}	31.3^{c}	21.1^{c}	22.7^{c}	21.2^{c}
Thigh	32.7^{a}	26.3^{abc}	32.3^{ab}	26.6^{abc}	31.3^{ab}	31.0^{ab}	30.0^{ab}
Linolenic (18:3)							
Breast	1.3^{ed}	0.8^{ef}	1.1^{de}	0.4^{gh}	0.2^{h}	—	—
Thigh	2.7^{a}	1.9^{b}	2.5^{a}	1.7^{bc}	0.7^{fg}	—	—
Arachidonic (20:4)							
Breast	6.4^{bc}	8.5^{ab}	9.0^{a}	8.0^{ab}	9.3^{a}	7.9^{ab}	7.7^{abc}
Thigh	4.9^{de}	4.8^{de}	5.8^{cd}	6.5^{cd}	4.5^{de}	3.1^{e}	2.9^{e}
Cerotic (26:0)							
Breast	7.7^{a}	5.5^{b}	2.9^{de}	2.4^{def}	3.5^{cd}	4.9^{bc}	5.2^{b}
Thigh	3.3^{d}	2.5^{def}	1.4^{f}	1.6^{ef}	2.3^{def}	1.4^{f}	1.0^{f}

[b] Means with the same superscript are not signifieantly different at 0.01 level within each fatty acid

significant regression coefficient in breast tissues only, and Table 18 shows no significant differences between means at ages 12, 16, and 20 weeks. This suggests variability in the phospholipid fraction of two muscle tissue types complementing the suggested changes of the neutral lipid fraction. The greater quantities of pentadecanoic (15:0),

arachidonic (20:4), and cerotic (26:0) in breast lipids may be due to higher concentration of phospholipids in that lipid fraction. The large array of different fatty acids found in these tissues provide the basis for the production of various carbonyls observed by Dimick and MacNeil (1970).

Fatty acid composition of Biceps femoris at three age periods are presented in Table 19. Similar to those in breast and thigh, Biceps femoris fatty acids represented by carbon chain length: double bond number of 15:0, 16:0, 18:0, 18:1, 18:2, 20:4 and 26:0 accounted for over 80 percent of all fatty acids. Greater concentrations of fatty acids 15:0, 16:0, 18:0, 20:0 and 20:4 were found in Biceps femoris muscle compared with thigh tissues. However, thigh tissues contained greater concentrations of 18:2. When considering those in greatest abundance, fatty acids of the Biceps femoris tend to follow similar patterns of breast fatty acids, (Table 15), suggesting similarities in intramuscular lipid fractions, or differences when intermuscular variability is included.

Most fatty acids from the Biceps femoris lipid fraction were significantly affected by age (Table 40, Appendix). Oleic acid (18:1) demonstrated the largest positive regression coefficient (Table 20 and Table 17) in all tissues. Major fatty acids 16:0, 16:1, 18:0 and 18:1 responded to age influences in similar directions in all tissues. Pentadecanoic acid (15:0), however, was negatively associated with age in thigh and Biceps femoris muscle tissues while a significant positive association with age was observed for breast tissues. Fatty acids from

Table 19. Fatty acids (%) of Biceps femoris muscle at various ages

Fatty Acid	Average	Age (Weeks) 20	24	28
8:0	0.12	0.16	0.08	0.12
9:0	0.18	0.14	0.05	0.28
10:0	0.08	0.07	0.05	0.11
11:0	0.25	0.23	0.08	0.45
12:0	0.39	0.32	0.12	0.72
13:0	0.25	0.41	0.07	0.28
14:0	0.81	0.75	0.53	1.16
15:0	3.10**	4.02	2.83	2.46
16:0	16.94*	15.71	17.95	17.16
16:1	2.32	1.74	2.40	2.81
17:0	0.84	1.50	0.47	0.56
18:0	12.65*	13.43	12.55	11.97
18:1	18.62	14.20	22.03	19.64
18:2	21.62**	24.57	29.68	27.62
18:3	0.57	0.57	—	—
20:0	2.20**	2.22	2.22	2.19
20:1	0.51	0.78	0.30	0.36
20:4	6.14**	7.73	5.85	4.95
22:0	0.76	0.14	—	1.39
22:1	0.79	0.65	0.36	1.38
22:5	0.92	1.55	0.48	0.74
22.6	0.87	0.81	1.12	0.67
24:0	0.58	0.72	0.49	0.55
26:0	2.23	1.75	2.81	2.13

* Significant at 0.05 level

** Significant at 0.01 level

Biceps femoris muscle lipid extract show similarities and differences to fatty acids of both breast and thigh lipid extracts. Biceps femoris fatty acid levels were significantly affected by muscle type with fatty acid 16:1 and 20:1 being exceptions.

Table 20. Relationship of Biceps femoris fatty acids with age

Fatty Acid	Regression Coefficients b_1[a]	b_{11}
14:0	.051***	.027*
15:0	-.195**	.026**
16:0	.182*	-.095**
16:1	.135**	-.008*
18:0	-.183*	.010
18:1	.652**	-.119**
18:2	.006	-.131*
20:1	-.052**	.017**
20:4	.348**	.031**
22.6	.064*	.028**
26:0	.047	-.054**

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

Part II

The eating quality of turkey is dependent on many factors, but currently, technological attempts to improve the turkey have found considerable market acceptance. The technological answer usually involves an addition of lipid material to increase the juiciness, possibly tenderness and flavor of the product. Without the injected materials, however, the eating quality of turkey is difficult to determine when selecting the item from a grocer's shelf. Aids to predict eating quality can be helpful to the turkey industry in both retail sales and raw material selection for further processing. Moreover, the understanding of methods and means to naturally improve eating quality of the turkey may further the progress of the poultry industry.

Because of the market acceptance of injected products, added lipid components are believed to benefit the eating quality of the turkey. Since the quantity of muscular lipid becomes increasingly important to eating quality, predictions of muscular lipid levels become valuable. Lipid levels in certain muscles have been shown to depend significantly on the age of the bird. Certain anatomical locations possess varying lipid levels which are age dependent; specific areas were explored for relationships to predict lipid levels of the muscle tissues. In this experiment, the sampled turkeys reached market weight at an earlier age (Table 21) compared with turkeys in Experiment I. From these birds, depth of skin from specified neck areas were measured and the results presented in Table 22 and Figure 6.

Table 21. Weight of tom turkeys at various ages

Age (Weeks)	Weight (kg)	Standard Error
12	5.51	0.182
16	8.60	0.324
20	9.98	0.385
24	13.03	0.297

Table 22. Skin thickness of turkeys at various ages

	Location	
Age (Weeks)	Beard (mm.)	One inch from Beard
12	18.96[a]	33.88[a]
16	38.18[a]	51.73[a]
20	48.09[a]	92.39[a]
24	1095.84[b]	2479.16[b]

[a] Means with the same superscript are not significantly different at 0.01 level

Skin thickness increased as turkeys advanced in age, with a most dramatic increase appearing at 24 weeks of age. This suggests that rapid lipid accumulation in skin located near the beard occurs after 20 weeks of age. From Table 41 (Appendix), significant effects of these measurements with age are reported. Both measurements evidenced significant linear and quadratic regression coefficients reported in Table 23, and demonstrates the extent of lipid accumulation. Marion and Woodroof (1965) reported variation in skin lipid with anatomical location in broilers suggesting the need for constant observation areas.

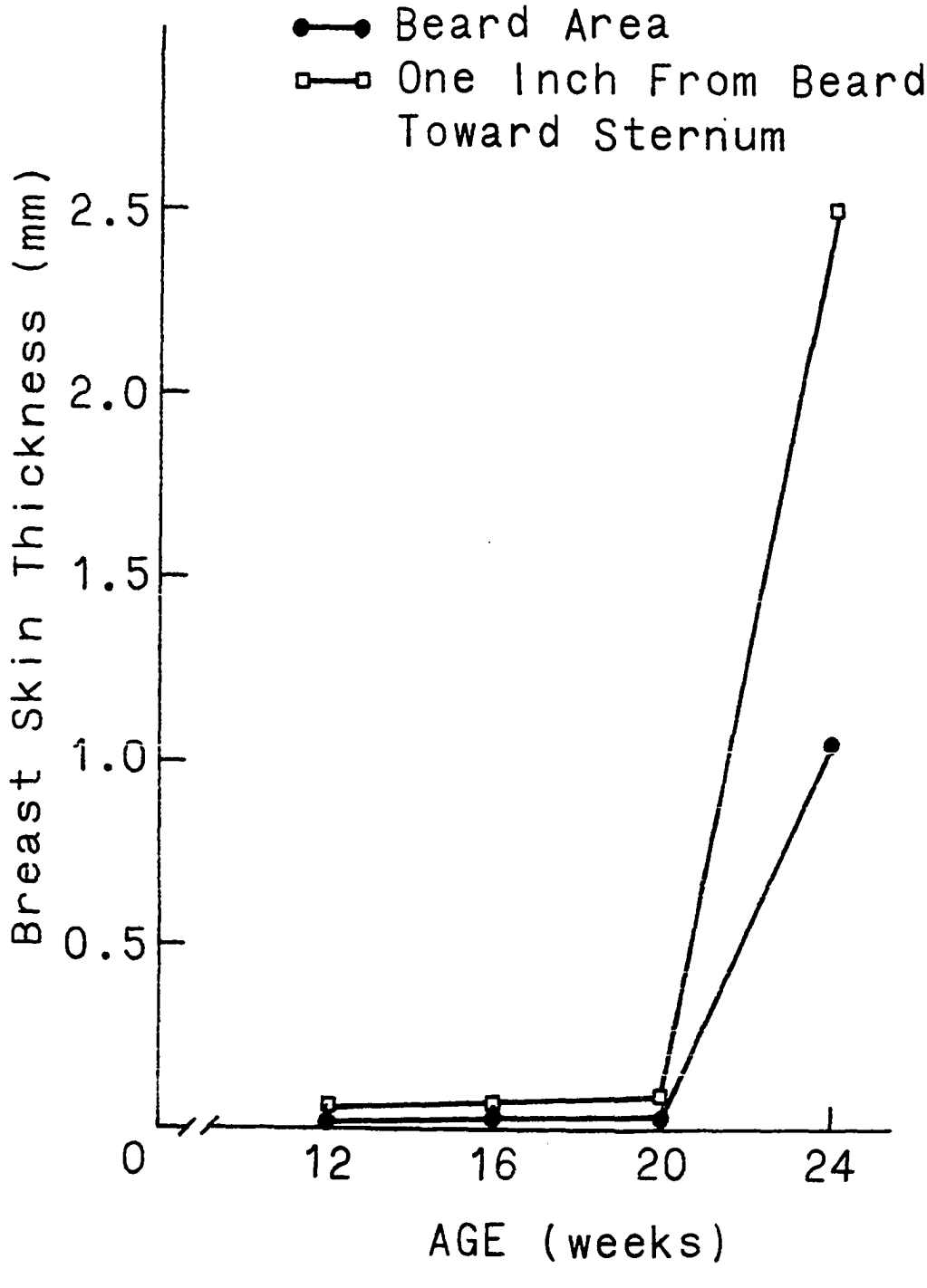

Figure 6. The effect of age on thickness of neck skin

Table 23. Regression coefficients of skin thickness and age

Skin thickness	Regression Coefficients b_1[a]	b_{11}
Beard area	6.982**	0.699**
Beard area - 1 inch toward keel	17.163**	2.132**

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coeffic coefficient

** Significance at 0.01 level

Proximate analysis

Proximate analysis of muscles was studied at various ages. In Table 24 and Figures 7, 8 and 9, the proximate analysis means are reported for each muscle at each age. Certain proximate analysis parameters are shown to vary with age and muscle type (Table 42, Appendix). Lipid content is significantly affected by age and muscle type. At 24 weeks of age, lipid content means of the Biceps femoris and Gastrocnemius were not significantly different, but both means were significantly different from similar means of the Pectoralis major and wing. Pectoralis major, Biceps femoris and Gastrocnemius lipid content-age coefficients were significant and positive in direction (Table 24). Biceps femoris and Gastrocnemius associations appeared larger than Pectoralis major regression coefficients, lending support to the idea of greater lipid increases in individual leg muscles than in breast muscles. Wing tissues showed a significant and negative association between age and lipid content. The Pectoralis major and

Table 24. Proximate analysis of various muscles at various ages

Factors	Age (Weeks) 12	16	20	24
Lipid (%)				
Pectoralis major	0.87[f]	1.00[e]	0.91[f]	1.11[d]
Biceps femoris	1.54[d]	1.42[d]	1.69[c]	1.83[b]
Gastrocnemius	1.64[c]	1.60[d]	1.94[a]	1.84[b]
Wing	1.24[d]	0.95[ef]	0.88[f]	0.89[f]
Protein (%)				
Pectoralis major	24.18[a]	23.87[a]	24.13[a]	23.78[a]
Biceps femoris	19.96[c]	20.19[c]	19.61[c]	19.78[c]
Gastrocnemius	19.86[c]	19.84[c]	19.04[c]	19.33[c]
Wing	21.91[b]	22.31[b]	22.05[b]	22.16[b]
Moisture (%)				
Pectoralis major	73.77[e]	73.33[e]	73.37[e]	74.22[de]
Biceps femoris	77.09[a]	76.09[abc]	76.38[ab]	76.44[ab]
Gastrocnemius	76.82[ab]	77.03[a]	76.85[ab]	76.97[ab]
Wing	76.00[bc]	75.10[cd]	75.35[c]	76.03[bc]

[f] Means with the same superscript are not significantly different at 0.01 level (Duncan's multiple range test)

wing muscles were quite similar in lipid content, and these muscles contain significantly lower lipid quantities than the Biceps femoris and Gastrocnemius (Table 24). Although significant differences are reported, lipid concentrations of the Gastrocnemius and Biceps femoris were comparable (Figure 7). Lipid content of the Pectoralis major and Biceps femoris was comparable to previously reported data; however, thigh tissues averaged greater quantities of lipid from the individual

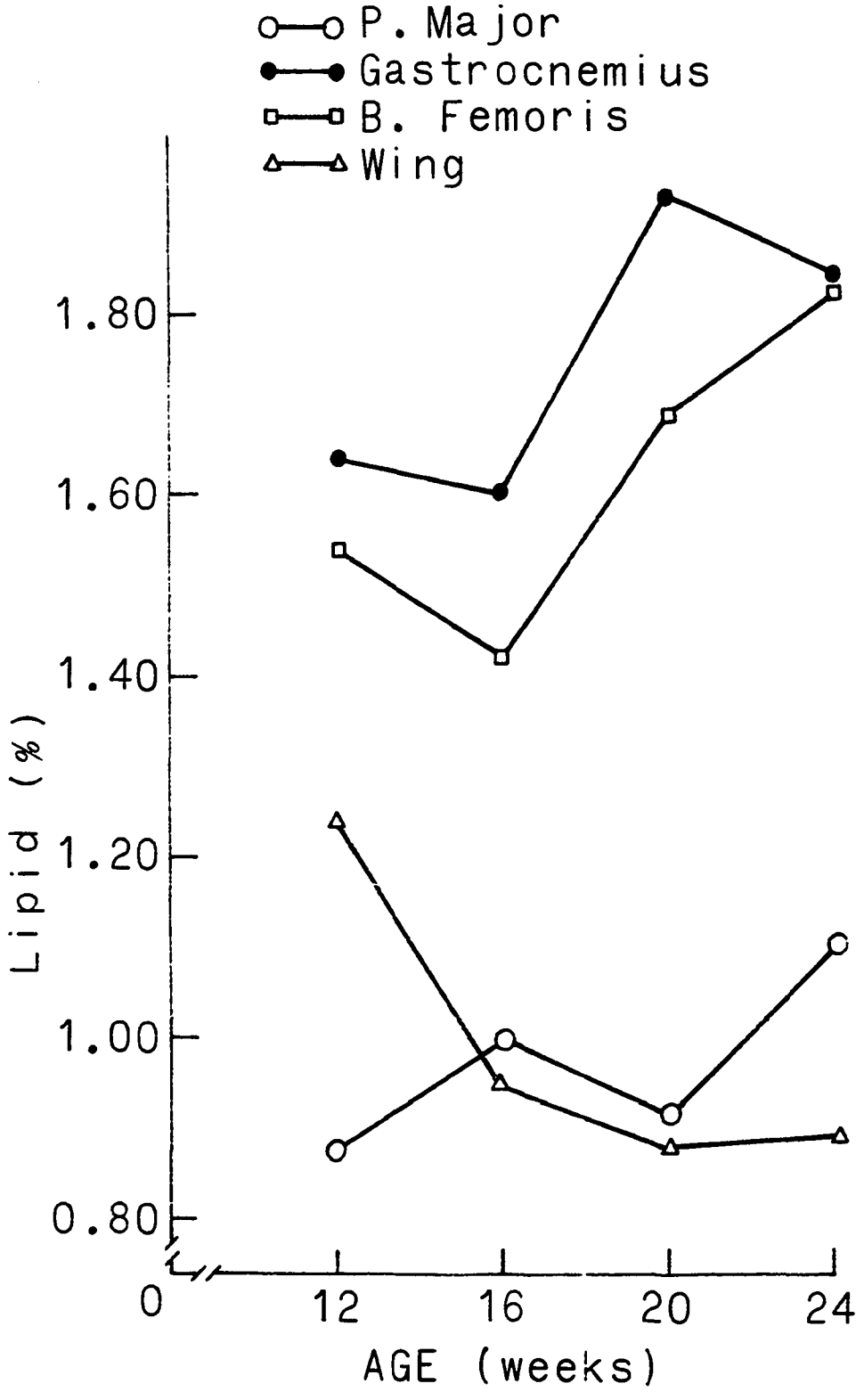

Figure 7. The effect of age on lipid content in specific muscles at various ages

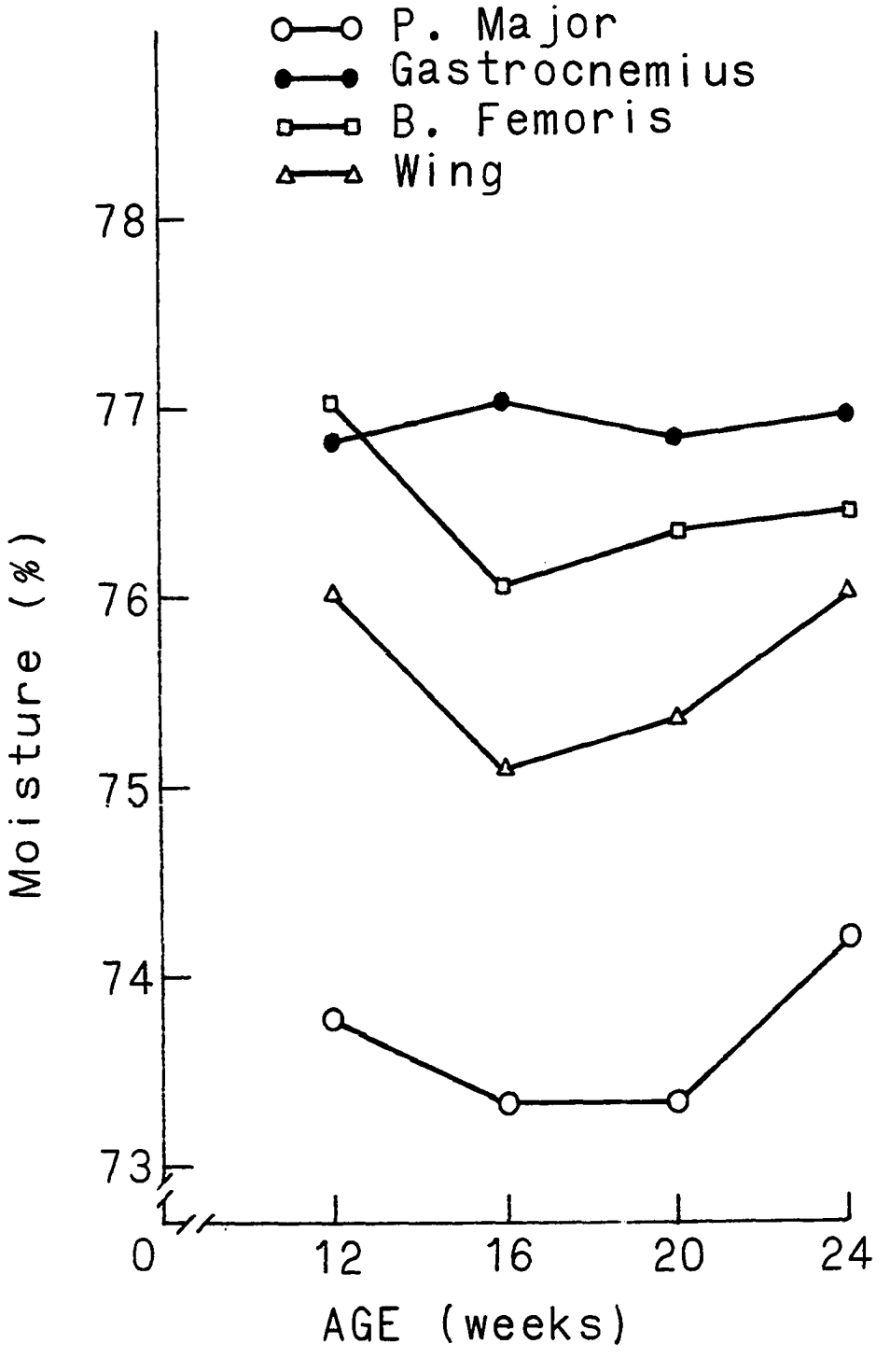

Figure 8. The effect of age on moisture content in specific muscles at various ages

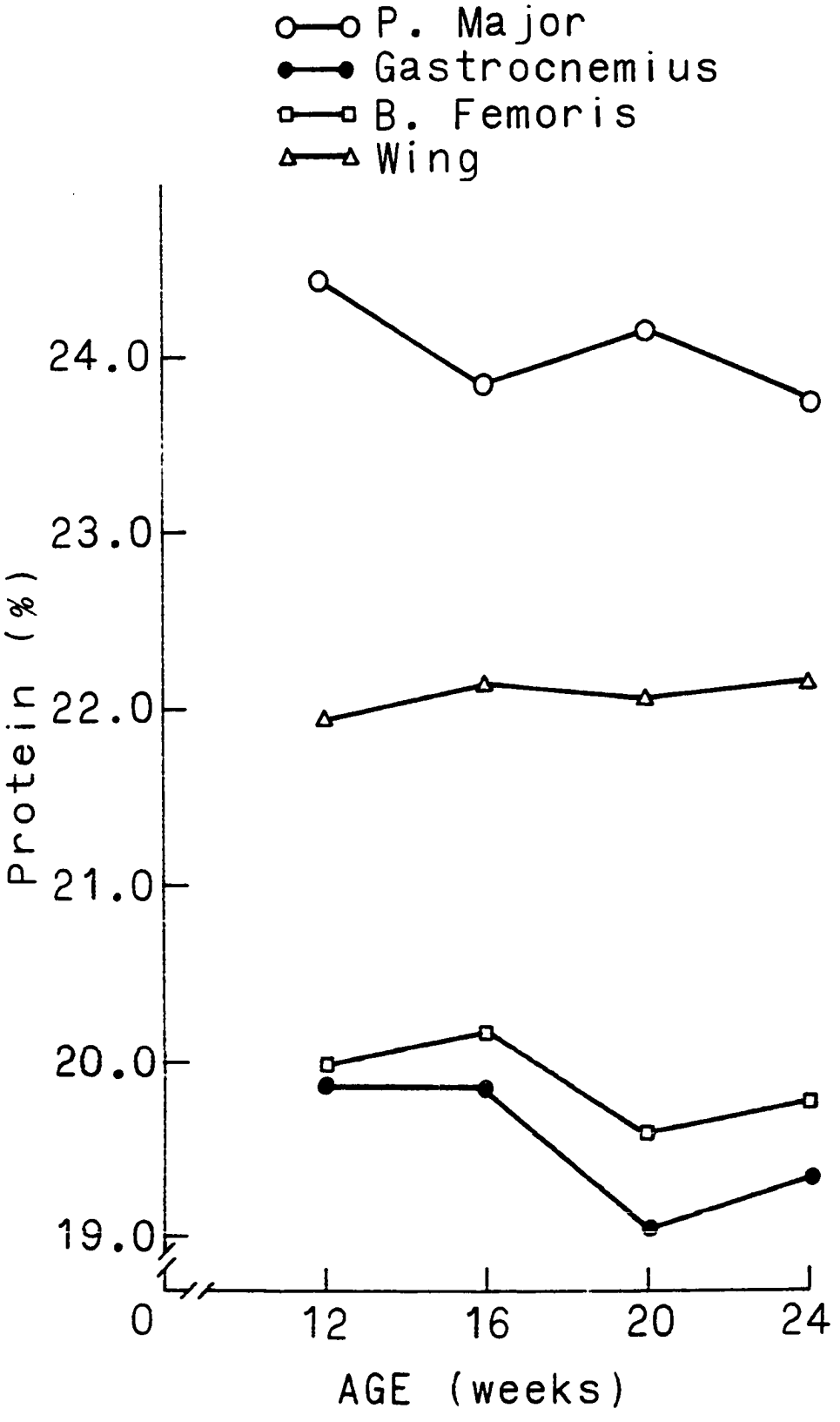

Figure 9. The effect of age on protein content in specific muscles at various ages

Table 25. Regression coefficients of proximate analysis parameters with age in various tissues

Factor	Regression coefficients b_1[a]	b_{11}
Lipid (%)		
Pectoralis major	0.015*	.001
Biceps femoris	0.029*	.004*
Gastrocnemius	0.024***	-.001
Wing	-0.028**	.005**
Protein (%)		
Pectoralis major	-0.023	-.001
Biceps femoris	-0.019	.001
Gastrocnemius	-0.059	.005
Wing	0.012	.005
Moisture (%)	0.035	.020**
Pectoralis major	0.035	.020**
Biceps femoris	0.035	.019*
Gastrocnemius	0.006	.002
Wing	0.008	.025*

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

muscles reported in this section.

Protein content did not change with age, but was affected by muscle type (Table 42, Appendix). Pectoralis major tissues contained the greatest concentration of protein followed by wing tissue. Protein levels of Biceps femoris and Gastrocnemius tissues were not significantly different, and they maintained a consistent level throughout these ages (Figure 8). A significant effect of age and muscle type on moisture levels was shown by the analysis of variance. However, no significant regression coefficient could be detected between moisture levels and age, and moreover, the Duncan's multiple range test showed no significant differences between means in any one tissue thus reducing the impact of the age effect suggestion. Between muscle tissues, Pectoralis major tissues possessed the lower moisture levels. Moisture levels of the wing were significantly greater than the Pectoralis major, and they were comparable to the moisture levels of the Gastrocnemius and Biceps femoris. Individual leg muscles did not show significant differences in moisture levels, but contained the greater proportions of moisture of the four muscles (Figure 9).

Phospholipid analysis

The distribution of total phospholipids for each age period and muscle tissue are presented in Table 26 and Figure 10. Phospholipid values showed no significant differences from the Duncan's multiple range test; however, the analysis of variance in Table 43 (Appendix) showed a significant age effect and a significant tissue effect. The age effect may be attributed to the wide distribution at 24 weeks of

Table 26. Phospholipid means of various tissues at various ages

Factor	Age (Weeks) 12	16	20	24
Total phospholipid				
Pectoralis major	672.92	714.96	615.16	474.50
Biceps femoris	654.03	672.61	662.70	691.10
Gastrocnemius	670.28	657.16	629.50	640.00
Wing	606.82	640.63	621.61	475.00
Percent phospholipid per total lipid				
Pectoralis major	66.88^{a}	62.53^{ab}	58.11^{abc}	42.89^{efg}
Biceps femoris	52.99^{bcde}	55.50^{bcd}	45.50^{defg}	39.44^{fg}
Gastrocnemius	48.60^{cdef}	46.98^{defg}	37.78^{fg}	36.82^{g}
Wing	53.61^{bcde}	63.61^{ab}	59.95^{ab}	53.91^{bcde}

[a] Means with the same superscript are not significantly different at 0.01 level

age. Phospholipids, expressed as a percent of total lipid (Figure 11), showed significant age, tissue type and interaction effects. Biceps femoris phospholipid (Mg/gm tissue) and wing phospholipid (%) were shown to have a non-significant regression coefficient, however, the Biceps femoris phospholipid regression coefficient appeared large. Phospholipid means presented in Table 26 are similar to values reported in the previous section. On a percentage basis, however, Pectoralis major phospholipid percentages appear lower than those in Table 7, whereas phospholipid concentrations of the thigh appear in greater quantities in Table 26. Biceps femoris and Gastrocnemius phospholipid

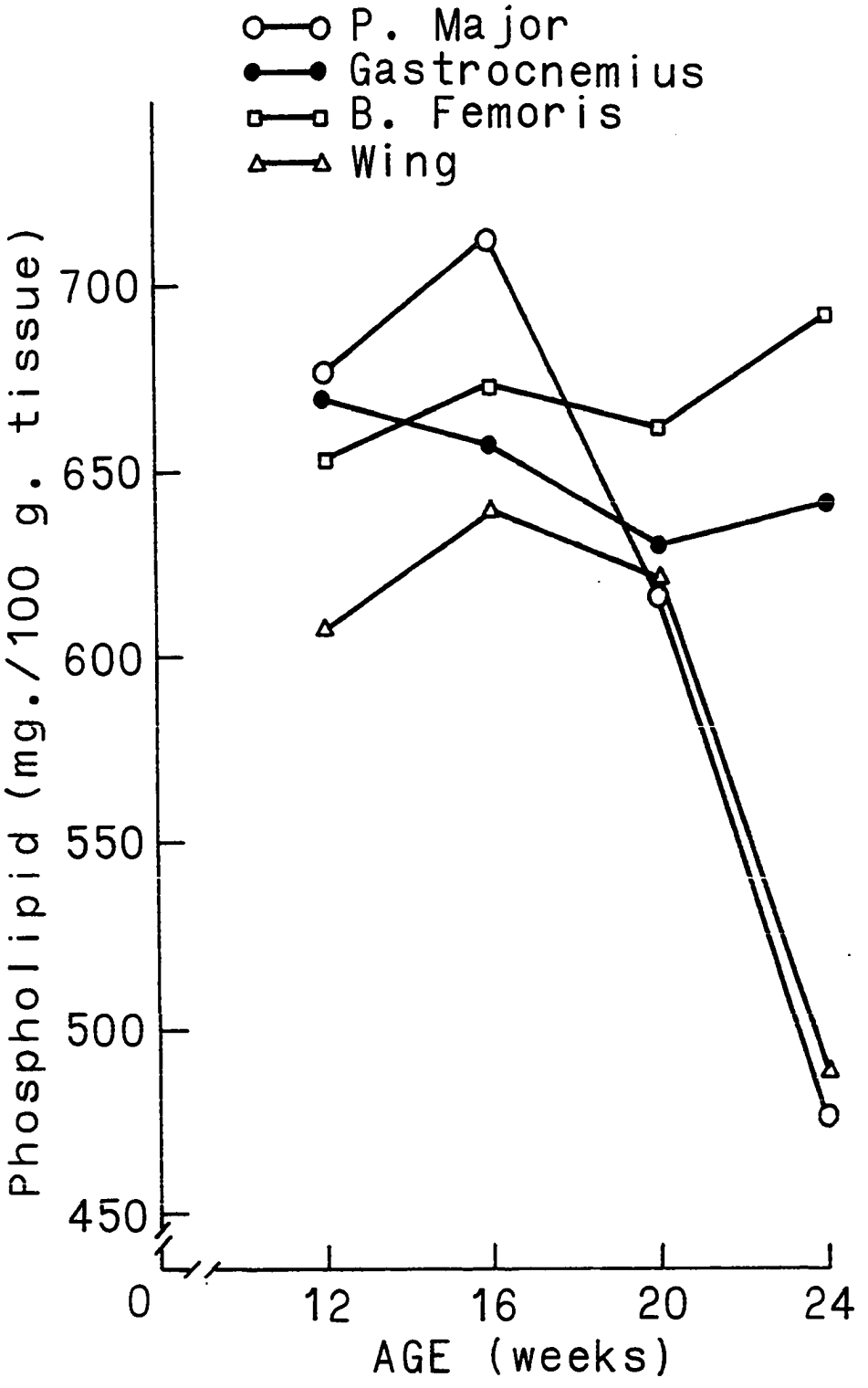

Figure 10. The effect of age on phospholipid content in specific muscles at various ages

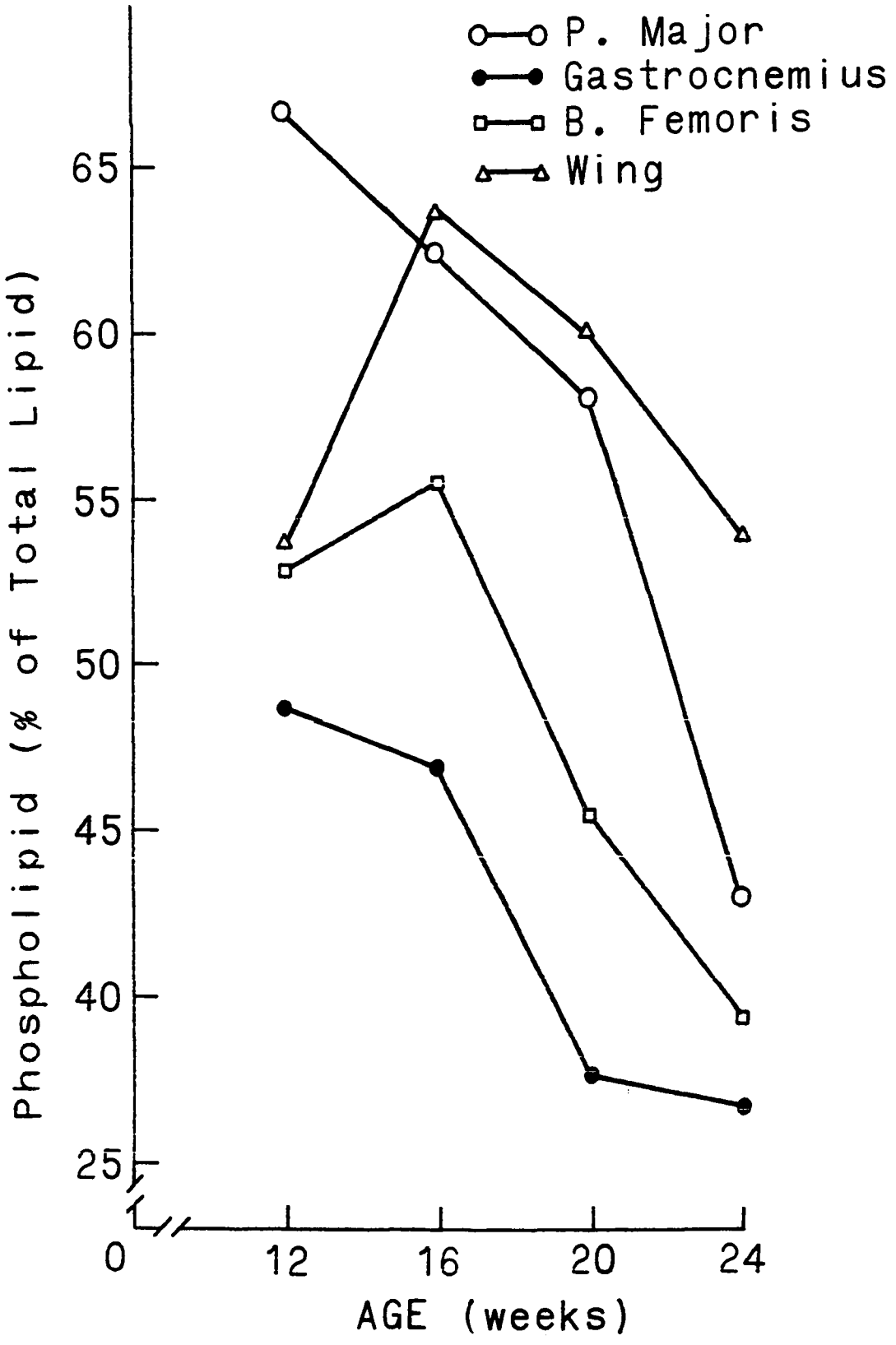

Figure 11. The effect of age on phospholipid content, as a percent of lipid, in specific muscles at various ages

concentrations were not significantly different within ages nor were breast and wing phospholipid concentrations, 12-week data being an exception. Wing and Pectoralis major had the greater concentrations of phospholipids.

Table 27. Regression coefficients of phospholipid factors and age

Factors	Regression coefficients b_1[a]	b_{11}
Total phospholipid		
Pectoralis major	-10.21**	-1.36**
Biceps femoris	-5.84	-1.33
Gastrocnemius	-9.73**	-.01*
Wing	-14.61**	-.05*
Percent phospholipid per total lipid		
Pectoralis major	-1.91**	-.17**
Biceps femoris	-1.27**	-.13**
Gastrocnemius	-1.08**	.03**
Wing	-0.05	-.24*

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level
** Significant at 0.01 level

Enzyme analysis

Lipid content has been shown to vary with turkey muscle and age, suggesting variable metabolic and physiologic need for lipid. In the domestic turkey, obvious differences in physiological activity occur among thigh, leg and breast muscles. George and Berger (1966) reported

metabolic differences for similar muscles of the domestic birds and related these differences to each muscle's physiological activity. Muscle metabolism, muscle physiological activity and muscle fiber type were reported to be associated by the same authors. The individual muscle's metabolic ability, or an alteration of its metabolic ability may be partly responsible for variable lipid content amoung muscles. This study was undertaken to further our knowledge of reasons related to lipid content variation.

Four enzymes of the three largest muscles were subjected to the histochemical techniques. Two enzymes responded to the histochemical techniques such that data could be obtained with confidence. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were not consistently detected in the muscles used, but would appear in a few instances as a weak response. Both enzymes are key enzymes in the pentose phosphate pathway, which is known to function for the generation of reduced nicotinamide-adenine-dinucleotide phosphate for lipogenesis. These enzymes have been reported in detectable concentrations of pigeon Pectoralis red fibers (George and Berger, 1966). But, these muscle enzymes of the domestic turkey weakly respond to the histological enzymatic techniques suggesting low concentrations and/or low enzymatic activity. Traces of 6-phosphogluconate and glucose-6-phosphate dehydrogenase activity have been reported in pork muscle, and no response from 6-phosphogluconate was observed in beef muscles (Bodwell et al., 1965) and Ogata and Mori (1964) reported low glucose-6-phosphate dehydrogenase activity in mouse muscle. With weak enzyma-

tic responses, lipogenesis becomes reduced and metabolic requirements become more dependent on incorporating the available lipids transported to the areas of need.

Histochemical responses to certain enzymatic activities consistently appeared in muscle sections from turkey Biceps femoris and Gastrocnemius. Examples of the histochemical responses for the two turkey muscle sections are presented in Figures 12, 13, 14 and 15. The fibers appearing darker in color stain positive for the specific active enzyme (Dawson and Romanul, 1964). Fibers stained positive appear randomly distributed in turkey muscle sections. Unlike turkey tissues, porcine Longissimus dorsi muscle fibers, staining positive for specific enzymes appear in clumps, as shown in Figures 16 and 17. In addition, porcine muscle tissue appears to have muscle fibers with greater specific enzyme activity. For each enzyme muscle histochemical response, percent positive fibers was obtained. Fibers which stained intermediately for an enzyme were counted as one-half of a positive fiber.

Means for lactic acid dehydrogenase (LAD) and beta hydroxybutyric acid dehydrogenase (BHBAD) enzyme responses for various muscles at different ages are presented in Table 28 and Figures 18, 19, 20 and 21. In the histochemical survey, detection of certain enzymes in specific muscles was not dependable or consistent and they are not represented in Table 28. From all enzyme responses, basic tissue differences were suggested in that enzyme responses from the Pectoralis major were never greater than enzyme responses of the Biceps femoris and Gastrocnemius. All enzyme responses of the Biceps femoris and Gastrocnemius reacted

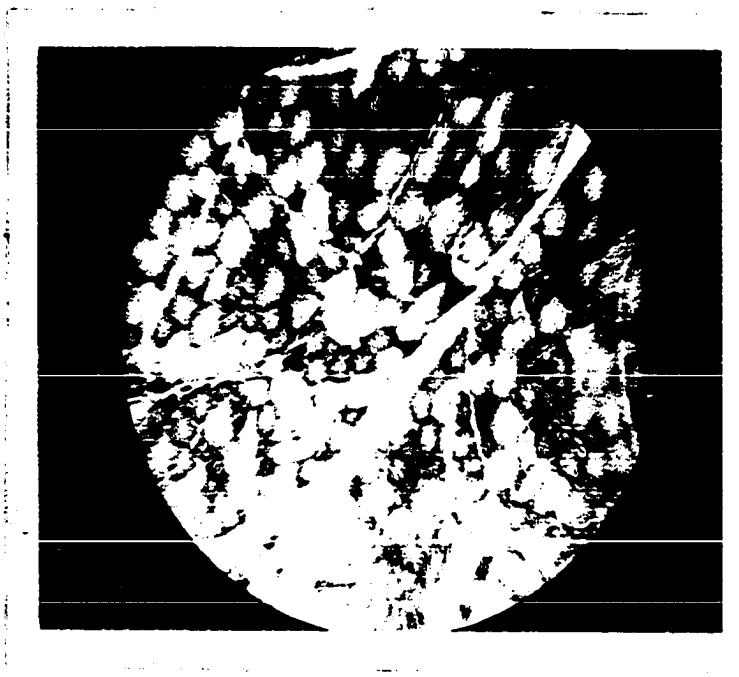

Figure 12. Cross section of turkey Biceps femoris and its histochemical fiber response to beta hydroxybutyrate dehydrogenase activity

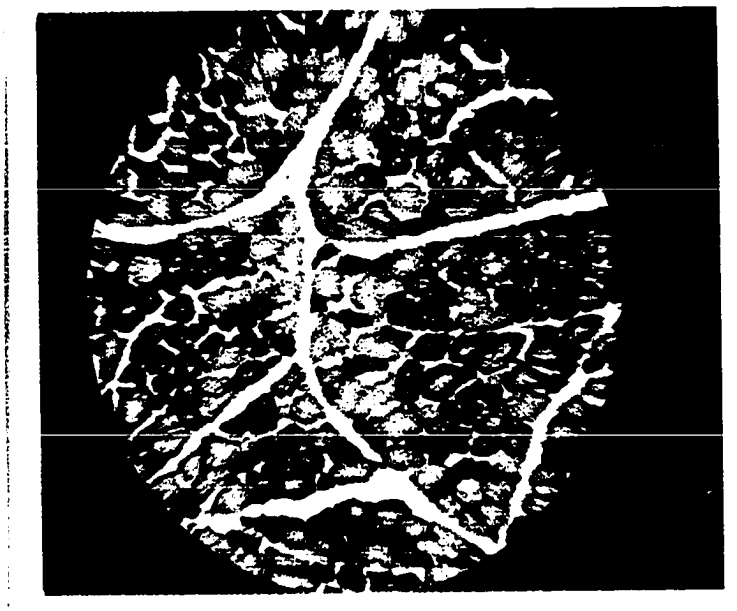

Figure 13. Cross section of turkey Biceps femoris and its histochemical fiber response to lactic acid dehydrogenase activity

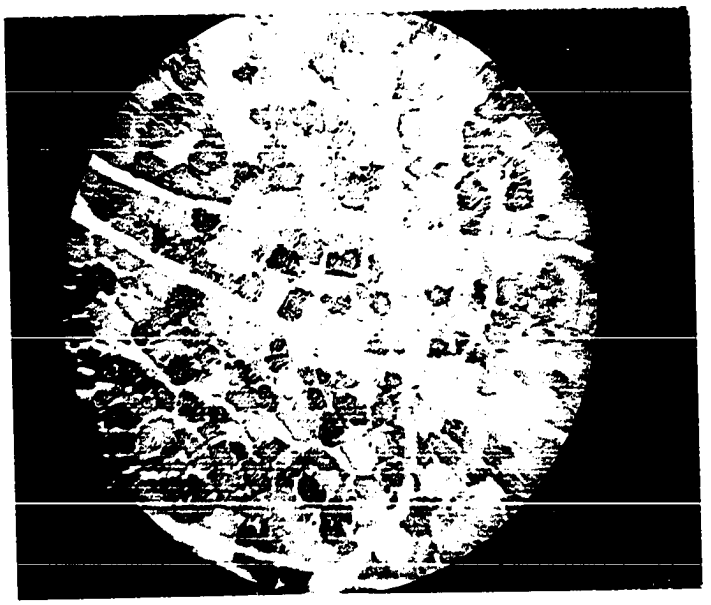

Figure 14. Cross section of turkey Gastrocnemius and its histochemical fiber response to beta hydroxybutyrate dehydrogenase activity

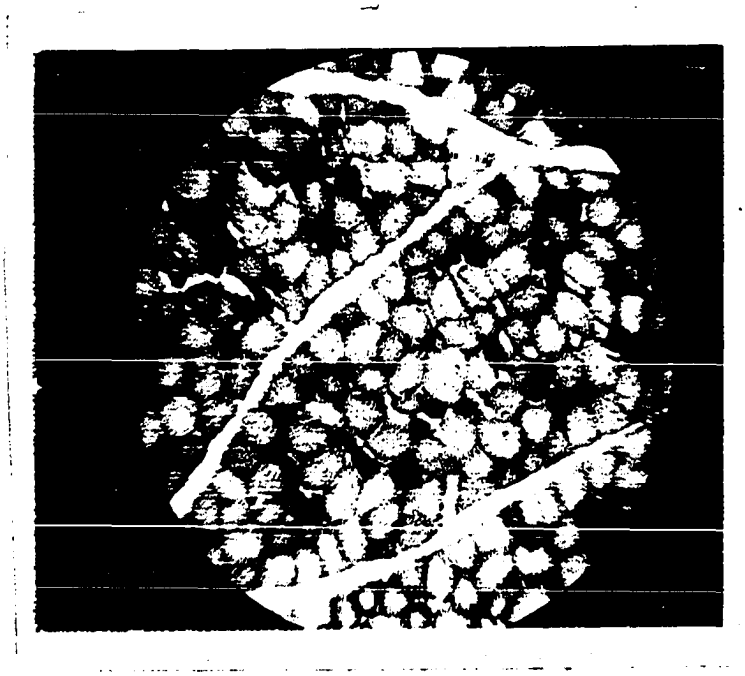

Figure 15. Cross section of turkey Gastrocnemius and its histochemical fiber response to lactic acid dehydrogenase activity

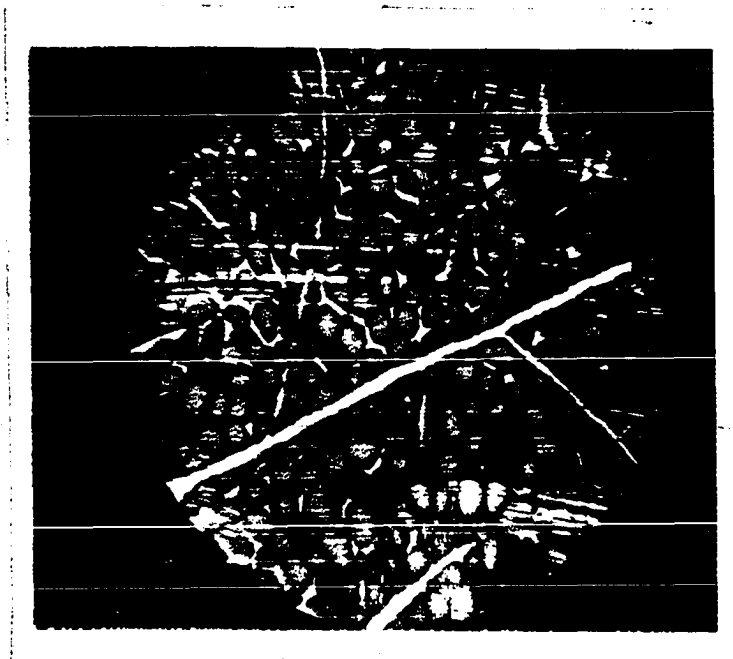

Figure 16. Cross section of porcine Longissimus dorsi and its histochemical response to lactic acid dehydrogenase activity

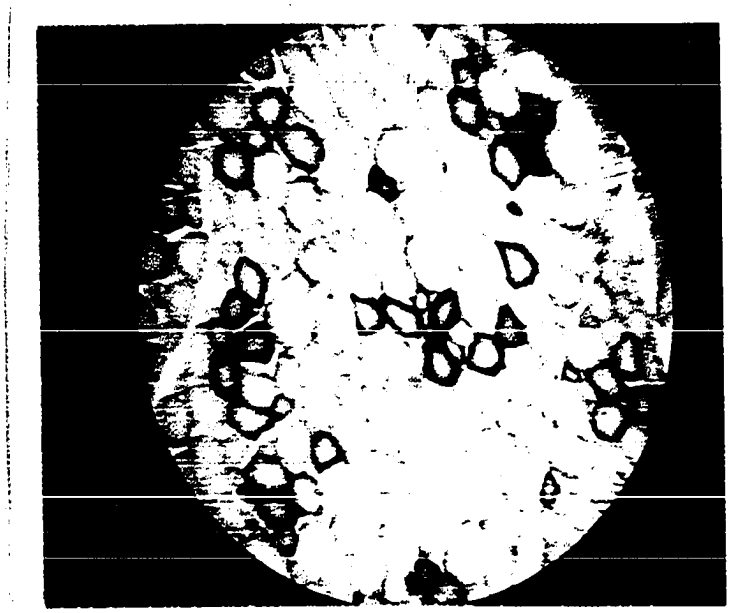

Figure 17. Cross section of porcine Longissimus dorsi and its histochemical response to beta hydroxybutyrate dehydrogenase activity

Table 28. Means of enzyme activity and percent fibers positive for enzymatic activity of various muscles at various ages

Factors	Age (Weeks) 12	14	20	24
Lactic acid dehydrogenase activity (Moles TPNH formed minute/mg. protein)				
Pectoralis major	—	128.4[b]	102.0[b]	98.09[b]
Biceps femoris	—	95.3[b]	113.0[b]	1240.00[a]
Gastocnemius	—	76.0[b]	116.0[b]	1211.00[a]
Beta hydroxybutyrate dehydrogenase activity (Microliters Oxygen/hour/10 mg. mitochondrial protein)				
Pectoralis major	1.74[de]	1.72[e]	2.28[bcde]	1.99[cde]
Biceps femoris	2.65[abc]	2.61[abcd]	3.04[ab]	2.46[abcde]
Gastrocnemius	2.16[cde]	2.67[abc]	3.21[a]	2.66[abc]
Percent fibers positive for lactic acid dehydrogenase				
Pectoralis major	—	—	—	—
Biceps femoris	47.62[a]	40.39[a]	51.75[a]	52.18[a]
Gastrocnemius	43.10[a]	48.39[a]	49.44[a]	48.41[a]
Percent fibers positive for beta hydroxybutyrate dehydrogenase				
Pectoralis major	—	—	—	—
Biceps femoris	30.45[a]	42.72[a]	44.33[a]	45.83[a]
Gastrocnemius	31.57[a]	43.11[a]	42.27[a]	44.71[a]

[b] Means with the same superscript are not significantly different at 0.05 level (Duncan's Multiple Range Test)

similarily, or at least not significantly different, within age periods. Both enzyme responses showed some change in activity at 24 weeks of age in the individual leg muscles, while small changes were noticed in the

breast muscles. The Biceps femoris and Gastrocnemius muscles are considered to be different from Pectoralis major tissues since leg muscles are physiologically more active and predominantly contain red muscle fibers, while white muscle fibers are believed to predominate in the breast tissues (George and Berger, 1966). Thus differences in characteristic fiber type, metabolism and composition of these tissues are of additional interest.

Enzymatic activity in muscle tissues is significantly affected by age (Table 44, Appendix). Although histochemical enzymatic responses were not significantly affected by age, the enzyme activity of both enzymes increased as turkey age advanced. The large, positive, linear relationships of LAD activity with age are shown in Table 28, while low, yet significant positive linear regression coefficients are shown for BHBAD activity. Histochemical enzyme responses and age relationships were positive for each enzyme studied. A high degree of association appeared for BHBAD enzyme responses in Biceps femoris muscle. Turkey muscles demonstrated differences in enzymatic responses throughout age.

Tables 30,31,32 and 33 present the relationships of each parameter with another within muscle tissues with age. Pectoralis major, Gastrocnemius and Biceps femoris lipid content were positively associated with skin thickness and bird weight, whereas wing lipid content was negatively associated. Lipid deposition sequences in the growing turkey are suggested. Lipid content was negatively associated with percent phospholipid of total lipid in all tissues. Greatest degrees of association occurred in the individual muscles of

Table 29. Relationships of enzymatic factors with age

Factor	Regression coefficients[a] b_1	b_{11}
Lactic acid dehydrogenase activity		
Pectoralis major	-2.24	.31
Biceps femoris	142.946*	34.59*
Gastrocnemius	141.919*	33.02*
Beta hydrogenase activity		
Pectoralis major	.03***	-.01
Biceps femoris	-.01	-.01
Gastrocnemius	.05	-.02
Percent fibers positive for lactic acid dehydrogenase		
Biceps femoris	.03	-.01
Gastrocnemius	.13	-.13
Percent fibers positive for beta hydroxybutyrate dehydrogenase		
Biceps femoris	.82*	.003
Gastrocnemius	.25	.006

[a] b_1, linear regression coefficient; b_{11}, quadratic regression coefficient

* Significant at 0.05 level

** Significant at 0.01 level

the leg suggesting that quantities of lipids other than phospholipids were being deposited in these areas, substantiating the reduction of phospholipid concentration. Phospholipid content displayed no strong association with lipid content in the larger muscles, and intermus-

cular variability may account for the association noted in wing muscle. The two expressible forms of phospholipids were highly associated in all muscles but the wing, noting that phospholipids and lipid content responses resemble one another at certain ages.

Enzymatic responses appeared to have varying degrees of association with each other. The two chemical measurements of enzyme activity of both leg muscles were weakly associated, however, the two histochemical measurements of enzyme activity were strongly associated. This suggests that some enzymes may be distributed in muscle tissues in similar patterns while the extent of enzymatic activity may be variable. For example, LAD activity and its histochemical response in the Gastrocnemius muscle are negatively associated; Figures 18 and 19 show enzyme activity increased while histochemical responses declined in the growing bird. In contrast, BHBAD activity and its histochemical response was positively related in the same muscle suggesting both enzyme distribution and enzyme activity react similarily (Figures 20 and 21). Biceps femoris muscles appeared to possess little association between the enzymatic responses.

Figures 18,19,20 and 21 present specific muscle enzymatic responses at various ages. At 20 weeks of age, BHBAD activity declined (Figure 20) in the leg muscles while LAD activity drastically increased in the same muscle and at the same age period (Figure 18). Another change occurring in muscle tissues included the deposition of lipid (Figure 3 and 7). The occurrence of enzymatic and composit-

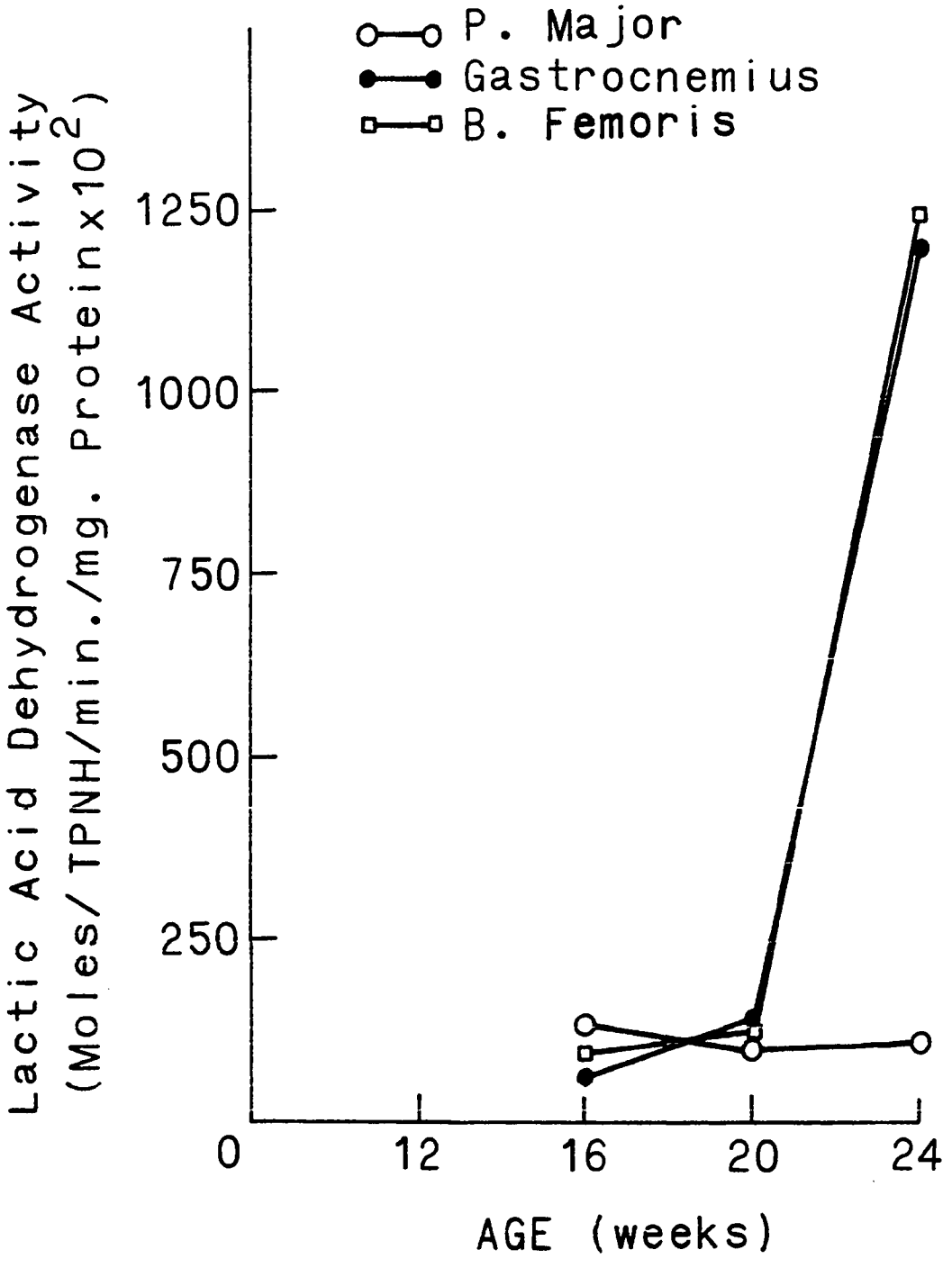

Figure 18. Effect of age on lactic acid dehydrogenase activity in turkey Biceps femoris and Gastrocnemius

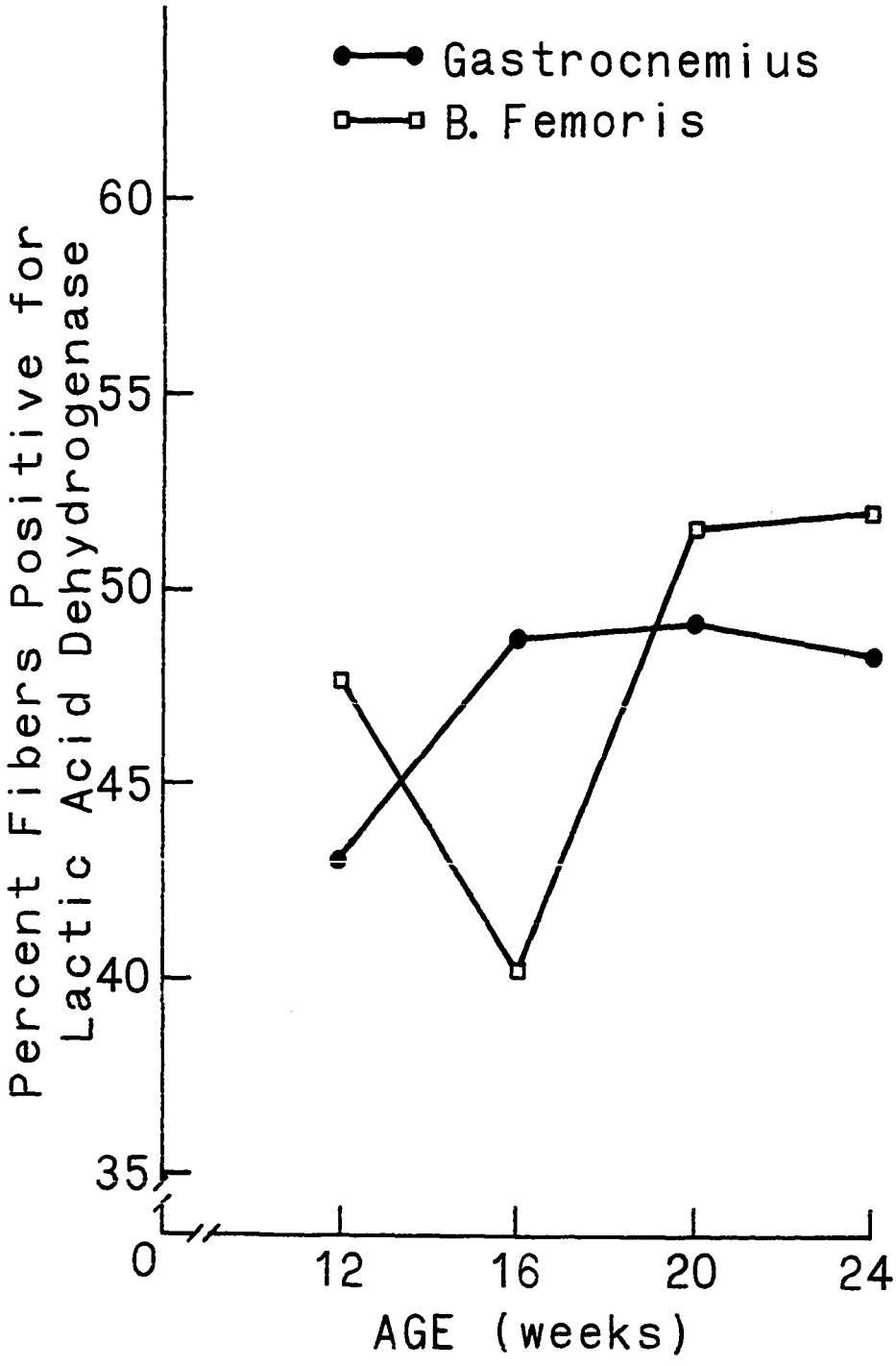

Figure 19. Effect of age on muscle fiber histochemical response to lactic acid dehydrogenase

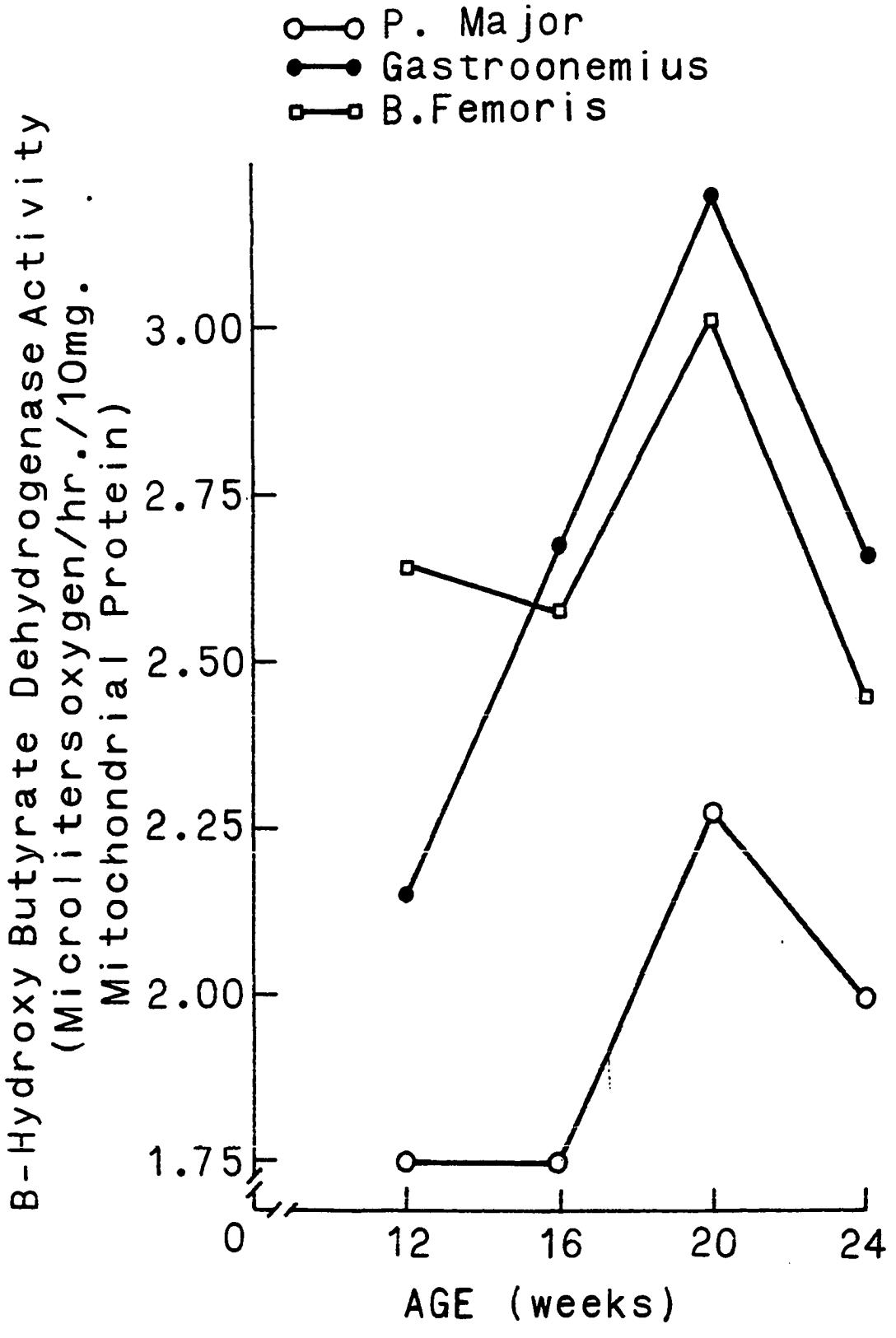

Figure 20. Effect of age on beta hydroxybutyrate dehydrogenase activity in turkey Biceps femoris and Gastrocnemius

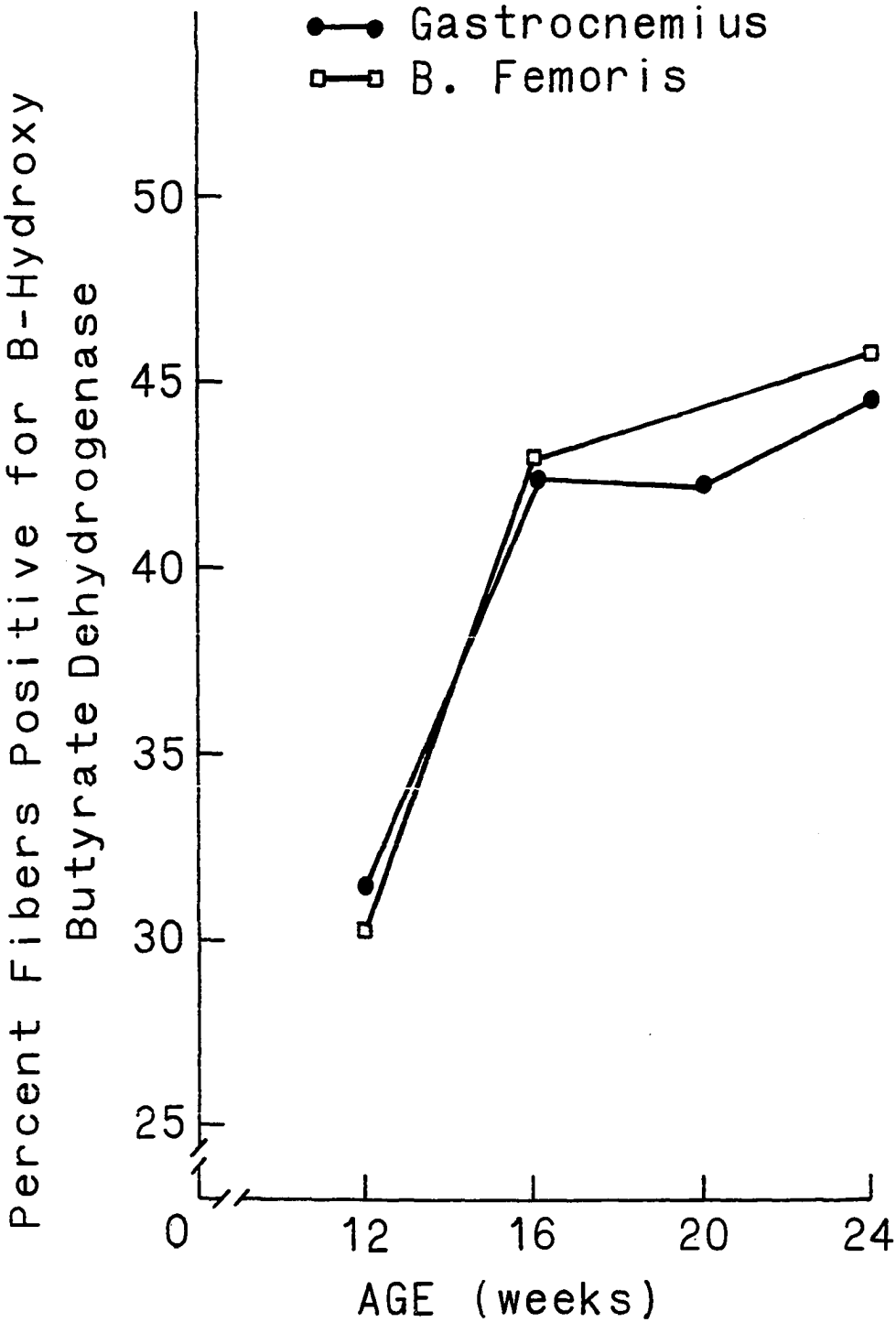

Figure 21. Effect of age on muscle fiber histochemical response to beta hydroxybutyrate dehydrogenase

ional differences infer differences in the capability of particular muscles to accumulate lipid as turkey age advances.

Table 30. Correlation coefficients of various parameters of wing tissue

	Skin Thickness		Lipid (%)	Total Phospholipid	Phospholipid/ Total lipid (%)
	Beard Area	One Inch Toward Sternum			
Bird Weight	.85	.79	-.62	-.77	-.10
Skin thickness					
Beard area		.86	-.38	-.64	-.26
One inch toward keel			-.30	-.52	-.20
Lipid (%)				.63	-.52
Total phospholipid					.34

Table 31. Correlation coefficient of various parameters from the Pectoralis major tissues

	Lipid (%)	Total Phospholipid	Phospholipid/ Total lipid (%)	BHBAD Activity	LAD Activity
Bird Weight	.52	-.52	-.82	.21	-.30
Skin thickness					
Beard area	.55	-.49	-.81	.16	-.12
One inch toward keel	.48	-.42	-.71	.33	-.11
Lipid (%)		.18	-.72	-.12	-.03
Total phospholipid			.54	-.22	.11
Phospholipid/total lipid (%)				-.06	.14
BHBAD activity					.01

Table 32. Correlation coefficients of various parameters from Gastrocnemius tissues

	Lipid (%)	Total Phospholipid	Phospholipid/ Total lipid (%)	BHBAD Activity	LAD Activity	%LAD	%BOH
Bird Weight	.36	-.60	-.59	.22	.50	.18	.25
Skin thickness							
Beard area	.20	-.61	-.47	-.10	.31	.15	.15
One inch toward keel	.40	-.51	-.55	-.10	.29	.20	.16
Lipid (%)		.01	-.82	.38	.06	.45	.16
Total phospholipid			.50	.09	-.07	.06	-.05
Phospholipid/total lipid (%)				-.25	-.11	-.36	-.21
BHBAD activity					.05	.10	.33
LAD activity						-.40	.07
Fibers positive for LAD (%)							.51

Table 33. Correlation coefficients of various parameters from Biceps femoris tissues

	Lipid (%)	Total Phospholipid	Phospholipid/ Total lipid (%)	BHBAD Activity	LAD Activity	%LAD	%BOH
Bird Weight	.39	-.24	-.58	-.08	.31	-.08	.39
Skin thickness							
Beard area	.51	-.25	-.63	-.28	.44	.09	.38
One inch toward keel	.57	-.29	-.68	-.14	.37	-.09	.33
Lipid (%)		.06	-.77	-.07	.26	.28	.13
Total phospholipid			.42	.09	-.17	-.29	.44
Phospholipid/total lipid (%)				-.06	-.33	-.42	-.31
BHBAD activity					-.09	-.13	-.18
LAD activity						.10	-.07
Fibers positive for LAD (%)							.30

SUMMARY AND CONCLUSIONS

As the male turkey approached market age, differences in types and quantities of lipid appear in various tissues. Neck skin thickness increased in the growing turkey, particularily between 20 and 24 weeks of age.

After 16 weeks of age, compositional changes occurred in leg tissues while few changes were found in Pectoralis major and wing tissues. Lipid accumulated after 16 weeks of age in leg tissues, however, phospholipid as a percentage of total lipid declined after this age. Cholesterol and cholesterol esters reached their highest level at 16 weeks of age in both tissues. Protein and moisture levels demonstrated no substantial change in certain individual muscles, however, thigh tissues moisture content declined significantly with age. Most fatty acids demonstrated an age effect, and many fatty acid changes occurred during this same age period.

The more physiologically active tissues were associated with greater quantities of lipid and lipid types. Cholesterol and cholesterol esters and phospholipid levels were consistently higher in leg tissues. But when expressed as a percentage of lipid content, these fractions were more concentrated in the less physiologically active tissues.

Numerous fatty acids were found in turkey muscle lipids, and most fatty acids were influenced by age and muscle type. Most abundant fatty acids found in turkey muscle lipid were those of chain length 15:0, 16:0, 18:0, 18:2, 20:4, and 26:0. Fatty acids commonly found in the phospholipid fraction were typical for that fraction at all age

periods; these responses support the importance of phospholipids as a class partly responsible for rancidity development. A large array of fatty acids was found, and they may account, at least in part, for the large variety of carbonyls reported in certain turkey products.

Phospholipid levels showed no significant variation with age, but comprised a decreasing percentage of total lipid as bird age advanced. Phosphatidylcholine and phosphatidylethanolamine accounted for approximately 50 and 22%, respectively, of the phospholipid fraction. Lysophosphatidylcholine (5%), sphingomyelin (10%) and other unidentified phospholipids comprised the remainder.

Between experiments, composition differences in muscle tissues appeared to be due to variability in sampling technique. By sampling a single muscle, intramuscular concepts (components inherent within a muscle) were expressed while both intra- and intermuscular variability was included in observations from thigh and wing tissues.

In addition to compositional changes after 16 weeks of age, enzymatic responses shifted. Since compositional and enzymatic response modifications occur at the same age, differences in lipid depositing capabilities among muscles are suggested.

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APPENDIX

Table 34. Analysis of variance for proximate analysis variables

Source of variation	df	Mean Square	F value
Moisture content			
Age	6	27.489	37.05**
Muscle type	1	256.507	621.25**
Interaction	6	3.015	7.30**
Error	63	0.413	
Protein content			
Age	6	19.984	27.65**
Muscle type	1	596.909	1018.43**
Interaction	6	0.592	1.01
Error	63	0.586	
Lipid content			
Age	6	4.686	23.88**
Muscle type	1	83.098	517.72**
Interaction	6	3.345	20.84**
Error	63	0.161	

** Significant at 0.01 level

Table 35. Least squares analysis of variance of phospholipids (mg/100g tissue) with age and muscle type

Source of variation	df	Mean Square	F value
Total phospholipids			
Age	6	22.972	18.77**
Muscle type	1	63.802	313.04**
Interaction	6	0.828	4.06**
Error	63	0.204	
Lysophatidylcholine			
Age	6	0.151	15.99**
Muscle type	1	0.001	0.01
Interaction	6	0.002	0.55
Error	63	0.003	
Sphingomyelin			
Age	6	0.491	21.17**
Muscle type	1	0.242	31.94**
Interaction	6	0.018	2.33*
Error	63	0.008	
Phosphatidylcholine			
Age	6	3.903	10.21**
Muscle type	1	11.858	131.24**
Interaction	6	0.343	3.80*
Error	63	0.090	
Phosphatidylinositol-Phosphatidylserine			
Age	6	0.359	12.95**
Muscle type	1	0.049	5.04*
Interaction	6	0.009	0.88
Error	63	0.010	
Phosphatidylethanolamine			
Age	6	2.418	14.93**
Muscle type	1	14.643	310.79**
Interaction	6	0.106	2.24*
Error	63	0.047	

* Significant at 0.05 level
** Significant at 0.01 level

Table 36. Analysis of variance for phospholipids (percent of total lipid) with age and muscle type

Source of variation	df	Mean Square	F value
Total Phospholipid			
Age	6	1392.969	14.48**
Muscle type	1	32422.902	503.14**
Interaction	6	362.400	5.62**
Error	63	64.441	
Lysophosphatidylcholine			
Age	6	23.071	11.23**
Muscle type	1	31.133	30.14**
Interaction	6	0.934	.90
Error	63	1.033	
Sphingomyelin			
Age	6	47.962	12.55**
Muscle type	1	20.529	10.75**
Interaction	6	3.067	1.61
Error	63	1.909	
Phosphatidylcholine			
Age	6	174.005	5.72**
Muscle type	1	108.077	13.47**
Interaction	6	12.431	1.55
Error	63	8.022	
Phosphatidylinositol-Phosphatidylserine			
Age	6	24.600	4.76**
Muscle type	1	68.236	29.20**
Interaction	6	1.777	.76
Error	63	2.337	
Phosphatidylethanolamine			
Age	6	65.315	4.20**
Muscle type	1	827.750	106.42**
Interaction	6	8.185	1.05
Error	63	7.778	

* Significant at 0.05 level
** Significant at 0.01 level

Table 37. Analysis of variance of Biceps femoris variables with age

Factor	df	Mean Square	F value
Lipid	2	2.06	18.41**
Error	27	0.11	
Protein	2	0.22	.30
Error	27	0.74	
Moisture	2	3.44	2.79
Error	27	1.23	
Phospholipid (mg/100 g meat)	2	10.46	8.94
Error	27	1.17	
Phospholipid (% of total lipid)	2	173.65	2.84
Error	27	61.16	

** Significant at 0.01 level

Table 38. Least squares analysis of cholesterol with age and type of muscle tissue

Source of variation	df	Mean Square	F value
Weight basis			
Muscle type	1	133474.90	253.30**
Age	5	6285.13	11.93**
Interaction	5	2958.35	5.61**
Error	108	526.95	
Percent basis			
Muscle type	1	436.32	127.46**
Age	5	155.55	45.44**
Interaction	5	11.95	3.49**
Error	108	3.42	

** Significant at 0.01 level

Table 39. Mean squares associated with the effects of age and muscle type on individual fatty acids

Fatty Acid[a]	Age[b]	Muscle type	Age x Muscle Interaction
8:0	0.096**	0.018	0.020**
9:0	0.089**	0.697**	0.013
10:0	0.40*	0.724**	0.040**
11:0	0.305**	0.278*	0.216**
12:0	0.592**	0.907**	0.061
13:0	0.323**	2.198**	0.087
14:0	1.634**	0.576*	0.332*
15:0	14.482**	310.397**	9.763**
16:0	97.596**	7.397**	33.902**
16:1	6.907**	6.032**	1.902**
17:0	5.389**	14.362**	2.084**
18:0	26.705*	57.999**	8.623*
18:1	241.589**	405.791**	40.033**
18:2	82.029*	2187.511**	18.203**
18:3	310.150**	71.400**	8.070**
20:0	11.225**	8.069**	2.291**
20:1	0.254*	3.792**	0.076
20:4	15.447**	428.120**	11.059**
22:0	1.369**	2.358**	0.419**
22:1	0.549*	1.945**	0.242**
22:5[c]	1.489**	18.361**	0.169
22:6[c]	0.451**	14.075**	0.347**
24:0	0.247*	10.082**	0.107
26:0	28.149**	247.141**	10.623**

[a] Carbon chain length: number of double bonds

[b] Significant at 0.05 (*) level, 0.01 (**) level

[c] Tentative identification

Table 40. Analysis of variance for fatty acids from thigh and Biceps femoris

Fatty Acid	Age	Muscle Type	Interaction
14:0	8.41**	0.06	0.30
15:0	24.42**	83.93**	2.71
16:0	11.67**	33.47**	0.63
16:1	9.85**	3.06	0.07
18:0	6.19*	40.19**	0.12
18:1	21.29**	51.14**	0.68
18:2	3.41*	25.94**	1.97
20:0	0.24	11.43**	0.20
20:1	12.61**	2.15	1.19
20:4	19.36**	79.25**	1.18
21:6	17.53**	15.79**	0.96
24:0	2.46	8.04**	0.90
26:0	8.28**	32.26**	29.75**

* Significant at 0.05 level
** Significant at 0.01 level

Table 41. Analysis of variance of skin thickness measurements

Factors	df	Mean Square	F value
Beard area			
Age	3	2251602.00	355.35**
Error	28	6336.18	
Beard area - 1 inch toward keel			
Age	3	1171594.00	60.38**
Error	28	194034.80	

** Significant at 0.01 level

Table 42. Analysis of variance for proximate analysis variables

Source of variation	df	Mean Square	F value
Percent Lipid			
Age	3	0.166	3.10*
Muscle type	3	5.390	100.93**
Interaction	9	0.202	3.78**
Error	112	0.053	
Percent Protein			
Age	3	0.804	1.18
Muscle type	3	138.107	202.53**
Interaction	9	0.484	0.71
Error	112	0.682	
Percent Moisture			
Age	3	2.504	5.32*
Muscle type	3	66.654	141.52**
Interaction	9	0.714	1.52
Error	112	0.471	

* Significant at 0.05 level
** Significant at 0.01 level

Table 43. Analysis of variance of phospholipid factors

Source of variation	df	Mean Square	F value
Total phospholipid			
Age	3	99995.50	14.72**
Muscle type	3	345713.80	50.90**
Interaction	9	6860.54	1.01
Error	112	6792.14	
Percent phospholipid per total lipid			
Age	3	1036.26	17.60**
Muscle type	3	1634.34	27.75**
Interaction	9	206.48	3.51**
Error	112	58.89	

** Significant at 0.01 level

Table 44. Analysis of variance of enzymatic factors with age and muscle tissue

Source of variation	df	Mean Square	F value
Lactic acid dehydrogenase activity			
Age	2	3343402.00	10.68**
Muscle type	3	1472676.00	3.74*
Interaction	6	1137360.00	2.89*
Error			
Beta hydroxybutyrate dehydrogenase activity			
Age	3	1.97	2.37
Muscle type	2	6.08	17.34**
Interaction	6	0.29	0.82
Error			
Percent fibers positive for lactic acid dehydrogenase			
Age	3	364.89	1.51
Muscle type	1	375.10	1.50
Interaction	3	222.92	0.89
Error			
Percent fibers positive for beta hydorxybutyrate dehydrogenase			
Age	3	27.79	0.05
Muscle type	1	1289.16	3.18
Interaction	3	674.15	1.66
Error			

* Significant at 0.05 level
** Significant at 0.01 level